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ANNUAL
REPORT

Division of Intramural Research Programs
National Institute of Mental Health

OCTOBER 1, 1985 - SEPTEMBER 30, 1986

VOLUME I
SUMMARY STATEMENTS

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Alcohol, Drug Abuse, and Mental Health Administration
National Institute of Mental Health
Division of Intramural Research Programs

ANNUAL REPORT

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NATIONAL INSTITUTE OF MENTAL HEALTH

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NATIONAL INSTITUTE OF MENTAL HEALTH

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ANNUAL REPORT
of the
Director, Division of Intramural Research Programs
October 1, 1985 - September 30, 1986
Frederick K. Goodwin, M.D.

Thanks to an unusually crowded schedule over the past several months, I have taken the liberty of extending the period actually covered by this, my fifth annual report as Scientific Director, to the end of calendar 1986.

The prospects for a productive 1987 are looking better than most of us would have predicted a year ago. Having made the most of a fiscally very tight 1986, caused by the sudden mid-year cuts required by the Gramm-Rudman-Hollings Act, the Program has emerged with some valuable lessons in belt tightening and with a modicum of new resources (albeit no new positions) to follow up exciting leads in AIDS, molecular biology, psychoimmunology and schizophrenia research. This continuity of intramural resources reflects the strong support of our program, especially at the Institute and ADAMHA level and also within the Department and in Congress. A key person in sustaining and enhancing support in the Institute's research mission is Dr. Shervert Frazier, whose sudden return to Harvard in December left us all with a sense of real loss. In his two years as Institute director, Dr. Frazier built new bridges between intramural and extramural programs, tapping the expertise of IRP scientists to help fill gaps in freshly reorganized headquarters branches. He also launched a major schizophrenia research initiative which is already reflected in a new Intramural branch. We will miss him.

As the search begins for a new Director, the Institute is in the able hands of Acting Director Dr. Frank Sullivan, who has a longstanding appreciation of intramural research; and -- for the first time -- an Acting Deputy Director drawn from the IRP. The recent naming of Dr. Rex Cowdry to the headquarters post is our loss and the Institute's gain. Dr. Cowdry distinguished himself over the past year as chairman of the NIH Clinical Center's Medical Board, tackling the difficult problems of budgeting resources for the world's largest research hospital. Dr. Dave Jimerson will serve as Acting Clinical Director during Rex's time at Parklawn.

Also in December, I was gratified to have been one of 44 senior Federal managers (one of 2 from DHHS) selected to receive the Distinguished Executive Award at a White House ceremony. I was genuinely surprised when President Reagan singled me out, along with three other honorees, for special mention. In his remarks, the President spoke of the importance of mental illness/neuroscience research to the Federal government's mission, apparently signaling heightened Administration recognition of our Institute's work.

Recognition from other quarters continued to be reflected in an impressive array of the most prestigious awards to IRP scientists. These included: Dr. Seymour Kaufman, election to membership in the National Academy of Sciences; Dr. Richard Wyatt, the Mental Health Association's McAlpin Research Achievement Award and the first Arthur P. Noyes Award in Schizophrenia from the State of Pennsylvania; Dr. Steven Paul, the Foundation's Fund Prize for Research In Psychiatry from The American Psychiatric Association and the 1986 Daniel Efron Award from the American College of Neuropsychopharmacology (ACNP); Dr. Daniel Weinberger, the Arthur S. Flemming Award for the ten best young Federal employees from the Downtown D.C. Jaycees; Dr. Robert Post, the ACNP's 1985 Efron Award; Dr. David Pickar, the National Alliance for the Mentally Ill's Young Scientist Award and the Commissioned Corps Commendation Medal; Drs. Joseph Zohar and Thomas Insel the A.E. Bennett Award (Research) from the Society for Biological Psychiatry; Dr. Karen Berman, the A.E. Bennett Award (Clinical); Dr. William Potter, the PHS Meritorious Service Medal; Dr. Michael Brownstein, the PHS Superior Service Award; Dr. Robert Cohen, the Commissioned Corps Commendation Medal; and Drs. Juan Saavedra and Steven Wise and Richard Staub and Mollie Strotkamp, the Administrator's Award for Meritorious Achievement.

To continue this tradition of scientific excellence, we periodically tap the expertise of our distinguished panel of outside consultants, the NIMH Board of Scientific Counselors. This year, we said "goodbye" to Dr. Daniel Freedman, who had served so ably as chairman of the Board. Danny will be succeeded by Dr. Arthur Prange. The incoming permanent Board members for l987 will include Drs. Lew Judd, Mike Goldstein and Bruce McEwen.

This year, as in past years, we have attempted to optimize research opportunities through programmatic and personnel rearrangements, as worked out in consultation with the Lab/Branch Chiefs, Associate Directors, the Board, and other panels of outside consultants. Last year's turnover in the basic science laboratories of Drs. Evarts, Costa and Kohn, allowed us to broaden our consideration of proposals for new programmatic thrusts. We convened an IRP "retreat "in early June, which included lab and section chiefs, to identify new areas of opportunity in the basic sciences where the IRP might recruit new staff. Focussing on scientific rather than structural issues, the day-long meeting discussed investigators doing the most exciting work in neuroscience and behavioral research areas who would complement our existing laboratories. A special focus was on those basic science areas where bridges to basic research could best be achieved.

A general consensus emerged favoring major new thrusts in developmental psychobiology, molecular mechanisms of memory and learning, molecular genetics and cognitive sciences. Three additional areas were identified for new recruitments, perhaps at a more junior level: psychoneuroimmunology, cellular neurophysiology, and protein chemistry and biochemistry. Committees were appointed in each area to review lists of optimal candidates and invite prospective recruits to present their work and get acquainted with the NIMH via a new speakers program. In each case, recruitment decisions would be based first on the quality of the individual's work and secondly on the ability of that work to interdigitate with areas already established in the IRP.

The area of cognitive sciences was also a point of focus for a distinguished ad hoc panel of psychologists brought in to advise us as to what were the most exciting and productive areas of psychological research today. The panel included Drs. Judith Rodin, Norman Garmezy and Michael Goldstein.

Among the first retreat-endorsed new thrusts is a new program in developmental psychobiology. A critical mass of basic animal investigations into the neuroanatomy and neurophysiology of development will soon be in place at our joint NIMH/NICHD animal research facilities in Poolesville, MD. We have recruited a senior investigator in this field who will collaborate with existing research teams led by Drs. Thomas Insel, Stephen Suomi and Steven Wise. Dr. Brent Stanfield, a highly regarded developmental neuroanatomist, studies the manner in which neurons interact to give rise to the complex organization of neuronal circuits.

Dr. Wise is moving the core of the Laboratory of Neurophysiology from Building 36 to Poolesville. His planned future studies on the prefrontal cortex there will attempt to link a growing understanding of the mechanisms underlying behavioral set with measurable activity of prefrontal neurons. Brain imaging studies at our William A. White (WAW) Building research center at Saint Elizabeths Hospital have linked impairment of behavioral set in schizophrenia to the frontal cortex. Future studies by Dr. Wise will also seek to unravel interactions between the cortex and striatum as they relate to activity of the dopamine system, which is known to be involved in schizophrenia and other neuropsychiatric disorders.

These developmental studies complement burgeoning investigations at WAW which are predicated on evidence that schizophrenia (and perhaps other forms of psychopathology) has its roots in brain systems which fail to develop normally. Drawing on existing primate studies of how the brain matures, Dr. Daniel Weinberger this past year elaborated on a developmental theory of schizophrenia that accounts for the characteristic delayed onset of the disorder in early adulthood and integrates other evidence from neuropharmacologic and neurophysiologic studies. Individuals at risk for developing schizophrenia may appear unimpaired in childhood because their immature brains can compensate for yet-to-be-discovered damage destined to affect functioning of the prefrontal cortex and related areas. It is only after these areas and related, stress-sensitive dopamine systems mature that symptoms develop.

Dr. Weinberger, Dr. Steve Paul and myself served on the NIMH Schizophrenia Task Force which is responsible for formulating the Institute's new schizophrenia initiative. As part of this heightened posture. The creation of a new Clinical Brain Disorders Branch, under Dr. Weinberger at WAW, provides a concrete reflection of our commitment to schizophrenia research. Created from elements of the existing Neuropsychiatry Branch and the Laboratory of Preclinical Pharmacology, the new branch brings to bear a range of technologies in search of causes and new treatments for this most devastating and least understood of the major psychiatric illnesses. In the coming year, single photon emission tomography will be added to the Branch's growing armamentarium of brain imaging techniques. In addition, Dr. Emanuel Casanova, a top flight neuropathologist, has been recruited to head up an expansion of the existing brain bank within the Clinical Brain Disorders Branch. It is being developed into a national resource for studies of schizophrenia and related disorders.

With the prospect of relocating our Saint Elizabeths operation to the NIH campus still years away, and faced with the imminent transfer of the Hospital to the District of Columbia, we have had to plan for the transition of the WAW facility into a freestanding research hospital. These preparations were made, over the past year, by Clinical Directors Drs. Rex Cowdry and Lew Bigelow in cooperation with Jim Pittman. In the past, the 60 patient care and support personnel of WAW's three inpatient units were employees of Saint Elizabeths Hospital. Later in 1987, they will be transferred to the IRP, increasing our total paid workforce to about 600 people. To acquaint Bethesda-based programs with the expanding resources and exciting research at the Saint Elizabeths outpost, we "invited" all lab and branch chiefs to a tour of WAW last May. The visit generated considerable interest and at least a few specific collaborations resulted.

Similar in concept to the WAW brain bank is a new cell bank begun by Dr. Elliot Gershon and colleagues in the Clinical Neurogenetics Branch (CNG) this year. They are assembling a collection of cells, from well-diagnosed pedigrees with manic-depressive illness and schizophrenia, which will form the nucleus of a resource for studies using genetic probes. Both clinical and basic studies in molecular genetics were yet another high priority area identified at the retreat in which new recruitments were made in 1986. A new molecular neurogenetics unit under Dr. Edward Ginns has joined Dr. Steven Paul's Clinical Neuroscience Branch. Dr. Ginns and colleagues, who came to us from the NINCDS, have been working on strategies for gene therapy in Gaucher's disease, and are now expanding their interest to include psychiatric illness. On the more basic side of molecular genetics this year, we are very excited about the arrival of Dr. H. Okayama to Dr. Michael Brownstein's Laboratory of Cell Biology.

The CNG lost Dr. John Nurnberger this year to the University of Indiana. Dr. Wade Berrettini, also of the CNG, succeeds John as director of our outpatient clinic. Throughout most of this year, Dr. Gershon, like Dr. Seymour Kety before him, served part-time as science advisor to the ADAMHA Administrator and as interim Director of the ADAMHA Office of Science. The Agency has benefited from his sound scientific judgment during this transitional period. Again, ADAMHA's gain is IRP's loss: Dr. Gershon's announced successor in the post, Dr. Bernard Grosser of the University of Utah, will have to resign from our Board of Scientific Counselors when he assumes the job this Spring.

Other IRP scientists who have been helping out part-time at Parklawn include Dr. Lavonne Brown, who has provided professional support for child mental health grant programs and, along with me, helped Dr. Frazier in his role as chair of the Secretary's Task Force on Youth Suicide. Among full-time transfers, Dr. Richard Nakamura has formally joined the extramural Neuroscience Branch and Dr. Lana Skirboll the ADAMHA Office of Science.

Fortunately, the traffic has not been all one-way. Just in time to help us manage a tight budget squeeze, Dr. Ted Colburn recruited computer management systems specialist Tom Cleary from Parklawn. He showed us how to quantify and institute new budget procedures. We began using our newly acquired computer capacity to do more careful budget projections and planning. For example, we can now integrate both the "other objects" and the personal services components of research projects in our budget analyses.

I chaired a committee of the NIH Scientific Directors on cost-cutting, while Dr. Carl Merril chaired a parallel effort within the IRP. If Gramm-Rudman had a bright side, perhaps it taught us some important lessons about where efficiencies could be achieved. Among innovative measures discussed over the past year have been the concepts of twinning and shared facilities. In twinning, a clinical research program is paired with a complementary basic program. For instance, we are in the process of strengthening Dr. Phil Gold's clinical neuroendocrinology program by incorporating Dr. Miles Herkenham's neuroanatomy unit. Dr. Gold's section is also assuming more clinical responsibilities for eating disorders studies. The sharing of facilities is being explored both within and across institutes. Dr. Mike Brownstein's Laboratory of Cell Biology now shares a peptide synthesizing facility with an NINCDS molecular genetics lab. Meanwhile, a growing number of mechanisms for supporting young investigators which do not use up FTE's -- NRC, NRSA, and the Hughes program -- is affording new flexibility in the management of scientific personnel.

This year, Dr. Peter Bridge returned to the IRP from 3 years with the ADAMHA Office of Science to serve as Assistant IRP Director for Special Projects. Dr. Bridge also serves as Deputy AIDS Coordinator for ADAMHA and as Co-Chairman of the PHS Subgroup on Behavior and Neuroscience, a component of the PHS Executive Task Force on AIDS. Science writer Bette Runck, formerly of the the NIMH Office of Scientific Information, joined our OD staff last summer and has been providing invaluable assistance in completing my book on manic-depressive illness. Ms. Runck has also been collaborating on other professional writing projects following the departure to an academic post in Seattle last summer of Dr. Peter Roy-Byrne, who served ably as my half-time special assistant.

As I noted earlier, the AIDS crisis is bringing in new dollars ($1.9 million for fiscal '87) and stimulating IRP research in psychoneuroimmunology. A potential new strategy for combating AIDS and other viral illnesses may even be emerging from basic research into the distribution of neuropeptide receptors in animal brain. Developed by Dr. Candace Pert and colleagues over the past year, the proposed new antiviral agent, called peptide T, is modeled on a tiny part of the AIDS virus that appears critical in gaining entry into cells. She has synthesized versions of the peptide which are resistant to metabolic breakdown and has shown that, even at low concentrations, they can inhibit viral infectivity, apparently by interfering with the attachment of the virus to the cell by occupying the relevant receptors on the cell membrane. In collaboration with immunologist Dr. Michael Ruff, who transferred to IRP from NIDR, she has also found that these same receptors are used by VIP (vasoactive intestinal peptide), which contains the peptide T sequence. Results of preliminary compassionate use of the peptide in Swedish AIDS patients have reportedly been promising. We currently have an IND application pending with the FDA to allow us to conduct a controlled study of the efficacy of this peptide in treating AIDS patients.

Among other noteworthy Intramural studies, Dr. Steve Paul and colleagues have demonstrated the first specific alcohol antagonist. This work promises to be of immense public health importance. Dr. Paul and colleagues have discovered a way of selectively blocking the early low dose anti-anxiety effects of ethanol in rats, that is, the effects which are clearly most important as reinforcers. Specifically, he has found that alcohol acts on the GABA/benzodiazepine/barbiturate receptor complex, stimulating uptake of chloride into the neuron. By injecting a benzodiazepine receptor antagonist (RO15-4513) into rat brain, Dr. Paul blocked alcohol's ability to stimulate this chloride uptake and its attendant behavioral effects. The findings help to explain the anti-anxiety, sedative-hypnotic and anesthetic properties of alcohol and may provide valuable insights into the mechanisms of alcohol tolerance and dependence. They also may lead to development of medications which could be used to treat alcoholism in much the same way that naloxone and naltrexone are used in the treatment of opiate addiction. Dr. Paul was one of only two scientists whose careers were featured in *Esquire Magazine's* 1986 Register of outstanding young Americans.

Given the obvious importance of animal research, it is depressing to have to report on the continued activities of animal rights extremists as they lay siege to the NIH campus and threaten to undermine political support for biomedical research, which is in fact one of the most humane institutions in our society. The NIMH IRP has a dual set of responsibilities in regard to animal research. First, we must strive to meet the high standards of care specified by the AAALAC. Second, we must help to educate the public about why we do animal research. I am proud to report progress on both fronts in 1986.

Working with the wise NIH leadership of Drs. Richard Wyatt and Robert Whitney, we have successfully negotiated for space in Building 14 to house our Laboratory of Neuropsychology (LN) in modern, up-to-standard facilities on an interim basis until a new neuroscience primate facility is completed. Planning money for the latter, "Building 49," to be located on the site of the parking lot southwest of the Clinical Center, is included in this year's budget. The LN hopes to move out of its obsolescent Building 9 quarters during the next year or so. Moving along with the Building 9 monkey colony will be our new veterinarian, Dr. Diane Blackmore, who, like her predecessor, Dr. Rob Werner, is adept at both clinical animal care and accreditation matters. Dr. Werner served admirably this year as chairman of the NIH committee that coordinates efforts to bring campus animal facilities up to AAALAC standards, and we wish him well in his new post in the IRP of the NIAAA.

The NIMH IRP is committed to conducting animal research that forwards our public health mission in the most humane manner possible. Yet, a recent incident brings home the fact that we must be especially vigilant in these times, for there are those who do not stop at deceit in their zeal to sabotage that mission. LN chief Dr. Mortimer Mishkin and colleague Dr. Elizabeth Murray appear to have foiled an attempt by an animal rights activist to infiltrate their primate lab by posing as a volunteer who claimed she was an admirer of their work and needed lab experience to begin a new career in neuroscience. Her suspicions raised by inconsistencies in this story, Dr. Murray began checking, and the facts did not add up. Later, this "research volunteer" surfaced in the news: she was arrested for demonstrating on the roof of a local primate research facility.

Dr. Steve Wise deserves special mention as a prime mover behind IRP activities addressing animal research issues. His commitment has helped to galvanize our public awareness efforts. Dozens of IRP scientists have invested time and ventured visibility in efforts to interpret the importance of animal research to the public over the past year. This message is often best conveyed by implication in news and feature stories about specific research findings. For instance, Dr. Mishkin drew heavily on his primate studies in a talk on the neuroanatomy of memory at an NIH Science Writers Seminar last spring. Similarly, Drs. Robert Post and Susan Weiss presented their rodent data on cocaine and kindling at an ADAMHA Science Press Seminar in August. Many other IRP investigators similarly implied the relevance of animal studies to human well being in media interviews too numerous to mention here.

However, the events of this past year have called for a more proactive approach, as well. Jules Asher, our public affairs specialist, has been coordinating the videotaping of IRP science to produce public information materials which explain the relevance of animal research by visually juxtaposing it with related clinical research. The footage also adds to our library of stock tapes to be handed out to television crews in lieu of their direct filming, thereby minimizing disruption to our labs and clinics. Moreover, it builds in a safety net regarding the sensitive issues of patient filming, allowing time for a careful informed consent process well in advance of any release (including time for second thoughts).

The raw footage has already proven effective in countering attempts by animal rights activists to manipulate television coverage of research. Last August, snippets of the tapes depicting animal studies by NIMH neuroscientists were provided to the American Psychological Association public affairs office. The APA had them duplicated and held them in reserve -- just in case. When animal rights groups demonstrated at the APA convention here in Washington later that month, the tapes were released to broadcasters covering the meeting. In most cases, this resulted in more responsible reportage than we would have seen if stations had only PETA-supplied tapes on hand.

PETA propaganda was again eclipsed by a combination of our tapes and an interview with one of our designated animal research spokesmen, Dr. Trey Sunderland, in a subsequent local TV news story about animal research. The NIMH proactive policy on the animal research issue was ultimately vindicated with publication in August of Katie McCabe's article "Who Will Live? Who Will Die?" in *The Washingtonian.* This article was the single most important factor in blunting the drive (orchestrated by animal rights groups) for a Congressional resolution condemning the NIH for the handling of the "Silver Spring Monkeys." It was also instrumental in heightening the awareness of health consumer groups of their stake in animal research, and in galvanizing a new coalition of such volunteer organizations for advocacy around this issue.

The newest of the mental health citizens groups participating in the above animal research advocacy coalition is one which we helped to inaugurate just last Spring. I was gratified to be among the speakers at the kickoff press briefing and Congressional reception for the National Depressive and Manic-Depressive Association, held on Capitol Hill during the week of the American Psychiatric Association convention here in May. The emergence of this grass roots affiliation of affective disorder patients is yet another sign that the stigma attached to serious mental illness is waning as research unearths more evidence of its essential similarity to other medical illness. Indeed, the NDMDA's charter requires that it be patient-directed. Katharine Graham's presence at the reception as guest-of-honor, as well as an impressive turnout among members of Congress, signals a growing awareness among opinion leaders and policymakers of the need for a major war on mental illness. An IRP-produced videotape of this event is available from our information office.

Also videoed for posterity is another unprecedented gathering: the first NIMH "All Hands Meeting" ever hosted by the IRP. As part of his policy of bringing together the Institute's intramural and extramural programs, Dr. Frazier this Fall bused some 300 Parklawn-based staffers to the Clinical Center, where, reinforced by hundreds more from IRP, NIMH nearly filled Masur Auditorium. Following presentations by Drs. Frazier, Goodwin and Cowdry, IRP senior staffers escorted the headquarters personnel on tours of the IRP labs of their choice. The response was enthusiastic and we hope to turn this into an annual tradition.

In fulfilling its civic duty, the IRP regularly calls upon some of our outstanding scientists to present their work at public lectures and smaller briefings for policymakers and interested citizens groups. This year, Dr. Rex Cowdry delivered an NIH Medicine for the Layman Lecture on depression, while Dr. Phil Gold presented a talk in the same series on stress hormones. Among the distinguished visitors who were briefed this year were DHHS Secretary Dr. Otis Bowen, White House science advisor Dr.John McTague, Assistant Secretary for Health Dr. Robert Windom, CDC director Dr. James Mason, FDA commissioner Dr. Frank Young, and NIH director Dr. James Wyngaarden. We also hosted a group of private citizens interested in mental health research, all of whom were especially impressed with the dedication of the Intramural scientists they met. Tours of the IRP were provided this year to a number of community groups, ranging from the likely -- the Philadelphia area NAMI affiliate -- to the seemingly not so likely: the Women's Leadership Program of the Defense Mapping Agency! Yet, as Dr. Rich Coppola reportedly told the latter group, the NIMH shares with them an interest in methodologies for the topographic mapping of roughly spherical objects. In any case, we believe such public education efforts are valuable in enhancing the climate for support of research and thank the many IRP scientists who graciously gave of their time.

Of course, the research enterprise demands that our scientists devote much of their away-from-the-lab time communicating with their scientific peers at professional conferences. IRP representation was especially prominent this year, with many of the major meetings being held in Washington, including both APAs, the ACNP and the Society for Neuroscience -- Dr. Mort Mishkin serving a distinguished term as president of the latter. Dr. Julie Axelrod gave an invited lecture on catecholamines at the psychiatric APA, while I presented a plenary talk at the ACNP.

IRP scientists played a major role in a number of Government-sponsored scientific meetings in 1986. These included : "AIDS: Challenge to Neuroscience, Psychology and Substance Abuse Research" in May; and NIH Conferences on Mechanisms of Physical and Emotional Stress in October and one on Alzheimer Disease last Winter. Our own Director's Conference Series, coordinated by Dr. Carl Merril, continued with rich and varied presentations on "Frontiers in Neuroscience" in October. For next year, we are initiating a monthly Research Rounds series and a series of informal workshops on the schizophrenia / neuroscience interface.

By virtue of our dual affiliations and the coincidence of institutional anniversaries, IRP scientists participated in some special NIH and NIMH scientific communications events this year. A two-day seminar at the National Academy of Sciences in June capped off a celebration of the 40th anniversary of the National Mental Health Act. Presenters included Drs. Mishkin and Wyatt from IRP. I chaired sessions on research on major disorders and also participated in a media roundtable at which Dr. Judy Rapoport presented her research findings on childhood obsessive-compulsive disorder and was critiqued by science journalists. In the Fall, a number of IRP investigators participated in a special "NIH Day" poster exhibition of work in progress held in the Clinical Center as part of the NIH Centennial. The NIMH IRP has been invited to develop materials to be included in NIH Centennial press packets and radio spots and, as one of the original six NIH Institutes, we have even been allocated space for a permanent exhibit in an NIH museum scheduled to open on the first floor of the Clinical Center next Summer.

Indeed, we can look back with pride on decades of remarkable scientific productivity, thanks to the stability of leadership, resources and critical mass afforded us through being part of both the ADAMHA and the greater NIH communities.

Annual Report of the Biological Psychiatry Branch, NIMH

October 1, 1985 - September 30, 1986

Robert M. Post, M.D., Chief

The Biological Psychiatry Branch (BPB) consists of several clinical and basic laboratory research programs dedicated to the elucidation and treatment of the psychobiological mechanisms underlying several types of mood and anxiety disorders. The clinical aspect of the Branch is centered around the 3-West Clinical Research Unit and two outpatient programs. Dr. Thomas W. Uhde heads the inpatient unit which primarily studies patients with unipolar and bipolar manic-depressive illness and panic anxiety disorder. In addition, he is Chief of the Unit on Anxiety and Affective Disorders and runs a large, multidisciplinary outpatient clinic for the study of patients with panic disorder. Dr. David Rubinow also heads up an outpatient program for the study and treatment of women with menstrually related mood disorder and is Chief of the NIMH Consultation-Liaison Service. Dr. Philip Gold, Chief of the Section on Clinical Neuroendocrinology, studies neuroendocrine aspects of patients with affective and anxiety disorders as well as patients admitted to other units in the Clinical Center with a variety of diagnoses including anorexia nervosa, bulimia, schizophrenia, and primary endocrinopathies such as Cushing's disease.

The basic laboratory component of the BPB consists of several groups headed by Dr. Paul Marangos (Chief, Unit on Neurochemistry), who studies neurotransmitter-receptor mechanisms such as those involved in adenosinergic and benzodiazepine neurotransmission as well as mechanisms at the calcium channel and related ion transport sites. He is also recognized as a leader in the study of neuron specific enolase which has now become an important clinical marker for patients with some types of cancer. Jitendra Patel, Ph.D., heads a group devoted to studying second-messenger aspects of synaptic transmission, primarily at the level of protein phosphorylation. Agu Pert, Ph.D., heads the Unit on Behavioral Pharmacology, studying the interaction of classical neurotransmitters and neuropeptide modulators in a variety of clinically relevant behavioral and pharmacological situations. Susan Weiss, Ph.D., directs the basic laboratory work of the Section on Psychobiology devoted to understanding the behavioral and biochemical consequences of repeated environmental, pharmacological, and electrophysiological stimulation in stress sensitization, cocaine sensitization, and kindling paradigms, respectively.

There is extensive interdisciplinary interchange among all of these clinical and basic investigative groups, although for the purposes of simplicity, the work of each group will be summarized separately.

Unit on Anxiety and Affective Disorders (Thomas W. Uhde, M.D., Chief)

Dr. Uhde's development of a systematic categorization of the life-course of illness in panic disorder has not only aided in the elucidation of the sequence of stages of the development of panic anxiety, but also in sub-categorizing the illness in relationship to possible differential pharmacological responsivity and underlying biochemical mechanisms. He has found that the onset of panic attacks, which generally occurs in adolescence or early adulthood, is almost uniformly the first expression of the disorder; then with repeated panic attacks, additional symptoms evolve including anticipatory anxiety and subsequent agoraphobia, as well as symptoms of major depression in approximately 50% of the patients. Life events or stresses of a peculiar type appear to be important in the onset of panic illness. Compared to controls (matched for age, sex, and degree of retrospective recall), 44 patients with panic anxiety disorder had more events "happening to them" and reported these events as associated with greater subjective distress, uncontrollability, undesirability, and causing lower self esteem. This tightly controlled study provides strong evidence that life events may play some role in the onset of panic disorder.

Panic-anxious patients, in addition to typical symptoms of anxiety and panic, also have a series of symptoms frequently experienced by patients with complex partial seizures - i.e., a variety of psychosensory and cognitive distortions in many sensory modalities. In fact, patients with panic anxiety disorder have as many of these symptoms as do those with psychomotor epilepsy. These data raise the possibility that some of the neural substrates involved in psychomotor epilepsy may also be primarily or secondarily dysregulated in the panic disorders.

Dr. Uhde has also found that patients with panic anxiety disorder do not have clinically enlarged ventricles, as measured by the ventricular brain ratio (VBR) on computer axial tomography scans (CAT scans). Not only do many patients with panic disorder tend to have small ventricles but those with the earlier age of onset and longer duration of disorder (age corrected) are the ones with smaller compared to larger VBR's within this patient population. However, those with longer histories of chronic benzodiazepine use were among those who had significantly larger VBR's.

Dr. Uhde has systematically compared clinical, biological, and pharmacological aspects of panic anxiety disorder to that of patients with affective illness, particularly in light of the common clinical responsivity of these two disorders to the tricyclic and monoamine oxidase inhibitor antidepressants. He has found several common endocrine abnormalities in these two syndromes, such as blunted ACTH response to CRF, blunted TSH response to TRH, and blunted growth hormone response to clonidine, yet patients have a dissimilar incidence of pathological escape from dexamethasone suppression (lower in panic anxiety compared to that in primary affective disorder). Moreover, he has found a differential clinical response to one night of total sleep deprivation; this procedure is associated with improvement in approximately 50% of depressed patients, but is not generally associated with improvement in mood or in anxiety level in panic disorder patients. In addition, 4 of the 12 panic disorder patients experienced panic attacks following sleep deprivation. Taken together, these data suggest that while patients with panic and depressive disorders share many biological and pharmacological responsivities in common, they can be differentiated by several probes such as response to dexamethasone and sleep deprivation. Parenthetically, studies of the calcium channel blocker verapamil suggest that this drug, which has recently been reported effective in some patients with manic-depressive illness, is without significant benefit when administered to panic anxiety patients.

Dr. Uhde has demonstrated that patients with panic anxiety have a differential response to high dose oral caffeine administration compared to normal volunteer controls. Nine of 24 panic anxiety patients experienced panic following oral administration of caffeine (480 mg), while none of 14 normal volunteers experienced extreme anxiety or panic at this dose. It is noteworthy, however, that two of twelve normal volunteer controls developed unequivocal panic attacks following the administration of a higher dose of caffeine (720 mg). These data suggest that high doses of caffeine can be panicogenic and that panic patients have a differential responsivity if not sensitivity to caffeine compared to normal volunteer controls. These data also correspond with earlier clinical evidence from this group based on self reports suggesting that panic patients had more difficulty tolerating caffeine-related products such as coffee and had given up drinking these agents because of CNS stimulation at a higher incidence compared to both affectively ill patients and normal volunteer controls.

Dr. Uhde has found that caffeine is associated with dose-related increases in cortisol, glucose, and lactate in normal volunteers and that each of these responses is significantly elevated in the panic patients compared with the normal volunteers. However, only plasma lactate levels are significantly higher in the panic patients who panic following caffeine compared with those who do not. The triazolobenzodiazepine drug, alprazolam, which has clinically significant antipanic effects, has also been shown to block caffeine-induced panic. None of the 11 patients infused with caffeine during alprazolam treatment developed panic.

Observations that caffeine could significantly increase plasma cortisol in high doses led Dr. Uhde and his co-workers to systematically evaluate the effects of caffeine on one of the most widely used neuroendocrine tests in psychiatry -- that of the dexamethasone suppression test. Following oral administration of 480 mg of caffeine or placebo in a single-blind design at 2:00-2:30 PM on the day following dexamethasone (1 mg p.o. administered at 11 PM the day before), they found a highly significant increase in plasma cortisol values at 4 PM; i.e., 5.3 ± 5.8 µg/dl compared to 2.3 ± 2.3 µg/dl, mean ± standard deviation, $p < .001$). Moreover, with the usual criteria of pathological nonsuppression (> 5 µg/dl), five of 38 subjects (13%) were found to be nonsuppressors on placebo, while this increased to 12 subjects (31%) following caffeine administration. These data suggest that caffeine and other dietary constituents may importantly influence this widely used test in clinical psychiatry. They also raise important questions of whether adenosinergic, benzodiazepine, or catecholaminergic mechanisms of caffeine may be implicated in this pathological hypercortisol response.

Glucose tolerance testing in panic-anxious patients has revealed both an important clinical finding and one of methodological significance. While panic patients experienced a variety of sympathetic responses associated with the late onset of mild hypoglycemia following the 1.5 gm/kg oral glucose challenge, they did not experience a high rate of panic attacks. These data thus suggest that hypoglycemia is not an important cause of panic attacks as is commonly assumed in the lay press, and, moreover, indicates that panic attacks do not occur nonspecifically following stresses or sympathetic activation, even one which reproduces many symptoms associated with panic attacks. These data thus indirectly support the interpretation that panic attacks induced by other chemical agents (such as caffeine, yohimbine, lactate) may provide important leads toward understanding the underlying biology of this disorder.

Dr. Uhde is studying behavioral and biological aspects of a colony of pointer dogs bred for normal and nervous behavior. The "nervous" dogs develop normally until eight to twelve months of age, when they begin to demonstrate marked behavioral impairments which resemble many aspects of pathological anxiety in man. Initial studies utilizing benzodiazepine-selective agents including the relatively pure antagonist Ro15-1788, suggest that an excess of endogenous benzodiazepine receptor stimulation is not causally related to this syndrome. Preliminary evidence suggests that there may be alterations in adenosine receptor binding in the nervous compared to normal dogs; other neurotransmitter and receptor alterations are being studied.

Section on Clinical Neuroendocrinology (Philip W. Gold, M.D., Chief)

The goal of this Section has been to extend current concepts regarding the physiology of neuroendocrine regulation in animal models, healthy volunteers, and patients with a variety of psychiatric and neuroendocrine disorders. Dr. Gold's work with the neuropeptide, corticotropin releasing hormone (CRH), reflects the successful implementation of these goals. Utilizing infusions of CRH (1 µg/kg) in several patient and control populations, he has clarified aspects of the pathophysiology of hypercortisolism in depression and anorexia nervosa. He has found that both of these psychiatric illnesses are associated with significantly blunted ACTH responses to CRH. In contrast, patients with the hypercortisolism of Cushing's disease show an increased ACTH response to CRH compared to normal volunteers.

In an updated series of 30 depressed patients compared to 29 patients with Cushing's disease, less than 25% of the patients showed overlap of their peak ACTH response to CRH, also suggesting the possible clinical utility of the CRH challenge in the differential diagnosis of these two illnesses. This differential response suggests that the locus of the abnormality in Cushing's disease may be at the level of the pituitary, where hypercortisolism of the illness is insufficient to blunt the ACTH response to CRH. In contrast, hypercortisolism in anorexia nervosa and in primary depression is sufficient to blunt the ACTH response to CRH, suggesting that the locus of the abnormality in these psychiatric syndromes may be occurring at or above the hypothalamus. These data are also consistent with the interpretation that CRH is hypersecreted in depressed patients and those with anorexia nervosa; indeed, this formulation is bolstered by the finding of increased CRH in the CSF of patients with underweight anorexia nervosa (studied with W. Kaye and D. Jimerson). Moreover, depression ratings in the weight-recovered anorectics correlated positively with the level of CRH in CSF. In depressed patients, CSF CRH was significantly correlated with the degree of blunting in the ACTH response to i.v. CRH.

Dr. Gold has postulated that there may be excessive stress-related secretion of CRH in depression and in Cushing's disease. In normal volunteers, he has demonstrated that there is dose-dependent pituitary-adrenal activation (presumably occurring because of CRH secretion), in response to greater levels of exercise. Moreover, he has found that administration of a continuous infusion of human CRH to normal volunteers reproduces the pattern of hypocortisolism commonly observed in non-depressed patients. However, the administration of frequent pulses or continuous infusions produced a smaller ACTH response than that seen after insulin-induced hypoglycemia. These findings suggest that stress-induced adrenal activation requires factors other than CRH and one of these may be vasopressin, another peptide studied by this investigative group.

In addition to these and a variety of other clinical studies, this group has studied neuroendocrine regulation in the laboratory using rat hypothalamic cell cultures and non-human primates. Utilizing this technique they have found that norepinephrine produces dose-dependent increases (rather than decreases as had previously been postulated) in CRH release. Acetylcholine and serotonin also stimulate CRH release, while activation of $GABA_A$ and $GABA_B$ receptors significantly inhibits serotonin-induced CRH release in a dose-dependent fashion. Glucocorticoids and ACTH also directly inhibit CRH release into the media in this preparation. A variety of related studies are also being conducted in the non-restrained primate. These reveal that dose-dependent inhibition of plasma ACTH is induced with alprazolam, diazepam, and baclofen.

Unit on Peptide Studies (David R. Rubinow, M.D., Chief)

To date, over 500 women have requested to participate in a project on the evaluation and treatment of menstrually-related mood disorder. Initial studies indicate that there are not significant differences in gonadotropins or gonadal steroids over the course of the menstrual cycle in patients with menstrually-related mood disorder compared with normal controls. Specifically, no deficiency in progesterone or alteration in the timing of its secretion was evidenced in this patient population, further casting doubt on the rationale for the widely-used progesterone therapy in the disorder. In fact, none of the first 14 patients treated in a placebo-controlled, double-blind fashion and followed longitudinally during progesterone therapy, improved with this treatment. It is also of interest that, pathological escape from dexamethasone suppression, frequently noted in primary depressive illness, was not observed in the dysphoric phase premenstrually in patients with menstrually-related mood disorder.

Studies of the nature of altered secretion of CSF somatostatin in patients with neuropsychiatric disorders are being conducted by Dr. Rubinow. Significant decreases in CSF somatostatin have already been observed to occur in a state-related fashion in depressive illness with normalization of values during recovery. In collaboration with Dr. T. Sunderland, Dr. Rubinow has observed significant decreases in CSF somatostatin in patients with Alzheimer's disease as well as in elderly depressed patients compared with an elderly control group. Values of CSF somatostatin are also lower in bulimic patients who are actively binging compared to when they abstain (studied with Drs. W. Kaye and D. Jimerson).

The interrelationships of cortisol hypersecretion and CSF somatostatin have been further documented. Not only is CSF somatostatin significantly lower in patients who show abnormal response to dexamethasone, but in collaboration with Drs. M. Kling and P. Gold, Dr. Rubinow has demonstrated that CSF somatostatin is significantly decreased in patients with pituitary-dependent Cushing's syndrome compared with controls. However, the nature of the interaction between somatostatin and cortisol remains to be clarified, particularly in light of the findings with Dr. O. Wolkowitz that volunteers treated with prednisone have significant decreases in CSF somatostatin, and the degree of decrease was associated with post-dexamethasone ACTH levels. Thus, while the regulatory relationships remain to be precisely delineated, it is clear that central somatostatin and CRH-ACTH systems are intimately related, with CSF somatostatin and CSF CRH levels highly correlated in a variety of patient populations including schizophrenics, bulimics, re-fed anorectics, Cushing's patients, and normal volunteers.

Alterations in brain somatostatin and its mRNA are being assessed during stress and following electrical kindling. Acute, unavoidable stress did not significantly alter somatostatin in a variety of brain regions in the rat. However, amygdala-kindled seizures (which have previously been reported to induce long-lasting increases in brain somatostatin) produced initial decreases in somatostatin mRNA in the hippocampus.

Unit on Behavioral Medicine (David R. Rubinow, M.D., Chief of NIMH Consultation Liaison Service)

A variety of clinical research protocols are conducted as part of the consultation liaison service investigating: 1) psychiatric symptomatology associated with medical illnesses and their treatment; 2) the role of previous psychiatric illness and stress on psychiatric and medical morbidity; 3) biochemical factors that may serve to predict cognitive and medical impairment; 4) the relationship of immunological function and dysfunction to alterations in mood and cognition.

Major findings include:

1) Treatment of chronic active hepatitis with alpha-interferon produced a high incidence (10 of 48) of reversable organic affective syndromes or delirium.

2) 60% of 44 cancer patients treated with interleukin-2 developed severe behavioral disturbance or cognitive impairment. These changes were closely linked in time to treatment with interleukin-2. While there were often unusual and severe symptoms, they were time-limited and resolved spontaneously. In order to follow up on possible mechanisms of the interleukin-2 effect, effects of interleukin-2 on the blood-brain-barrier are being studied. Initial results suggest a lack of effect on one measure of the integrity of the blood-brain-barrier, i.e., permeability of radiolabeled albumin.

3) Depression was more frequently associated with (and often the presenting symptom of) carcinoma of the pancreas (N = 12) compared with gastric carcinoma (N = 9). This differential association which has previously been reported in the literature did not occur in a second group of 16 patients with cancer of the pancreas and possible factors accounting for this discrepancy are being systematically explored.

4) Prominent mood changes are associated with alternate day steroid treatment in a sub-set of patients given this regimen for their lupus erythematosis. A similar pattern was also observed in a group of 12 normal volunteers treated for five days with a high dose of prednisone. Those who became most dysphoric had significantly lower levels of CSF somatostatin.

5) Patients with type 5 hyperlipoproteinemia did not display alterations in mood or cognitive function as a product of treatment with nicotinic acid. They were, however, characterized by an unusually high prevalence of history of psychiatric illness.

6) The relationship of panic disorder to the symptomatology of cardiac patients presenting with chest pain but normal coronary arteries is being studied. One of seven cardiac patients developed a panic attack during lactate infusion and four of the seven developed chest pain during either lactate or placebo infusion, suggesting the utility of this strategy in the psychological and biological assessment of this disorder.

Section on Psychobiology, (Robert M. Post, M.D., Acting Chief)

Studies are now being conducted to elucidate clinical and biological predictors of clinical response to the anticonvulsant carbamazepine, which has been developed as a new treatment for manic-depressive illness. Initial data suggest that many of the variables that predict less than robust response to lithium carbonate actually may be associated with clinical improvement during carbamazepine treatment of mania. In contrast to lithium responders, carbamazepine responders tend to be more severely ill at the outset, more dysphoric, more rapid cycling, and have a negative family history for manic-depressive illness in the first-degree relatives. The clinical profile of those who respond with an acute antidepressant response to carbamazepine include those who are initially more severely ill, those with good initial acute response to sleep deprivation, and those with more rapid cycling and less chronic illness. Degree of agitation or retardation as rated clinically or as assessed by motor activity does not predict carbamazepine response. While carbamazepine is associated with acute improvement in sleep in depressed patients, this occurs earlier than clinical improvement in mood, and the degree of sleep improvement is not significantly associated with the ultimate degree of improvement in depression. Improved sleep during carbamazepine treatment is occurring with an increase in total sleep time and slow-wave sleep without a significant reduction in rapid eye movement sleep.

In carbamazepine nonresponders, the addition of lithium produces more rapid improvement than is observed with lithium alone and appears clinically successful in one-half of the depressed patients studied to date, suggesting that lithium potentiation of carbamazepine may be similar to that observed with more routinely used tricyclic antidepressants. Side effects of lithium, which tend to override those produced by carbamazepine, include increases in white count, urine volume, and in plasma TSH levels.

Carbamazepine continues to show evidence that it may be useful either alone or in conjunction with lithium carbonate in the long-term prophylactic management of patients with manic-depressive illness. Many patients who have not previously done well on lithium carbonate appear to show excellent results with carbamazepine. The effectiveness of other anticonvulsants is being explored. We have observed some patients who respond to long-term prophylaxis with valproic acid (in combination with lithium) when they have not previously responded to lithium alone or to carbamazepine, suggesting that there may be differential response to anticonvulsants in affective disorders and that the anticonvulsants as a class of drugs may prove useful in the management of different phases of manic-depressive illness. This appears to be particularly the case in light of the data of others that clonazepam has a significant antimanic effect and alprazolam an antidepressant effect.

We have been engaged in a series of clinical and basic studies to better understand the mechanism of action of carbamazepine. In collaboration with S.R.B. Weiss, Ph.D., we have strong evidence that carbamazepine's anticonvulsant effects may be mediated, in part, through the peripheral-type benzodiazepine receptor. In contrast to diazepam, the anticonvulsant effects of carbamazepine are not reversed by the classical central antagonist, Ro15-1788. Conversely, the peripheral-type ligand, Ro5-4864, reverses the anticonvulsant effect of carbamazepine but not that of diazepam. The effects of Ro5-4864 on carbamazepine's anticonvulsant properties are themselves reversed by PK-11195, another ligand with relatively selective effects at the peripheral-type benzodiazepine receptor. These data, and a variety of other controlled studies, strongly imply that carbamazepine's anticonvulsant effects are differentially mediated from those of diazepam and involve mechanisms utilizing the "peripheral-type" benzodiazepine receptor mechanisms. These data are of particular interest in light of recent observations linking this receptor site to calcium channel fluxes as opposed to the central site which is linked to chloride fluxes. Calcium channel blockers have been reported by others to be effective in some patients with manic-depressive illness. Work by others also suggests that noradrenergic and sodium channel effects of carbamazepine may be important to its anticonvulsant profile.

While carbamazepine potently binds to adenosine receptors, chronic administration of carbamazepine paradoxically upregulates adenosine receptors in a fashion similar to other adenosine antagonists such as caffeine (studied with Dr. Marangos). Preliminary data suggests that the anticonvulsant effects of carbamazepine are not mediated through the adenosine receptor site. While Terrance and collaborators have suggested that $GABA_B$ mechanisms are involved in the antinociceptive effect of carbamazepine, we have found that these effects are unlikely to be related to carbamazepine's anticonvulsant efficacy. In contrast to the work of Terrance and collaborators on cell firing in the trigeminal nucleus, the inactive isomer of d-baclofen was unable to reverse the anticonvulsant effects of carbamazepine on amygdala kindling. Carbamazepine also significantly alters levels or activity of a number of neuropeptide systems. Carbamazepine significantly decreases somatostatin in CSF, it appears to act in part as a vasopressin agonist, it produces cortisol hypersecretion, facilitates opiate-induced running activity (but not antinociceptive effects), and decreases circulating thyroid levels without inducing clinically relevant hypothyroidism.

We continue to study the relationship of neurotransmitter alterations to the acute or longitudinal symptomatology of affective illness. For example, CSF norepinephrine is significantly increased in manic patients compared to depressed, euthymic, or normal volunteer control groups. Moreover, the degree of elevation is significantly correlated with ratings of manic dysphoria and anxiety. While we did not find CSF GABA significantly altered among groups of affectively ill patients, those with more severe illness, as measured by numbers of episodes and other indices, appear to have higher CSF GABA. Affectively ill patients with low 5-hydroxyindoleacetic acid may experience more severe depressions but those with higher levels have a greater frequency of episodes. These studies are among the first to attempt to elucidate the relationships of biological measures to the longitudinal course of manic-depressive illness as opposed to acute state-related fluctuations.

We have attempted to study the responsivity of the limbic system structures in man using the local anesthetic procaine. A variety of clinical and basic data suggest that this agent has some selectivity for limbic system structures. For example, in collaboration with R. Coppola, we have found that the drug induces increases in fast EEG activity (26-45 Hz) relatively selectively over the temporal cortex. The extent of this activation correlates with the degree of dysphoria produced. Patients experience a range of subjective mood changes from euphoria to dysphoria, as well as dose-related alterations in sensory and cognitive function. Patients experience illusions and hallucinatory-like phenomena in most sensory modalities in a fashion not dissimilar from that reported by Penfield and Gloor in their studies of direct electrical stimulation of temporal cortex and limbic system. In addition to these clinical and physiological effects, procaine also increases the release of cortisol and ACTH as well as prolactin, but not growth hormone (studied with Dr. M. Kling). Preliminary data, in collaboration with Drs. Rex Cowdry and David Gardner, suggest that the degree of EEG activation of temporal lobe structures by procaine is not correlated with subsequent response of patients with borderline personality disorder to the anticonvulsant carbamazepine. We have also not found a robust relationship between potential clinical markers of temporal lobe dysfunction, as revealed by interview-elicited ratings of the occurrence of psychosensory phenomena, and subsequent response to carbamazepine in patients with manic-depressive illness.

Dr. S.R.B. Weiss has conducted a series of studies designed to better understand the mechanism of action of cocaine. Initial studies have revealed that repeated administration of the same dose of cocaine leads to an increased degree of hyperactivity and stereotypy over time (behavioral sensitization). This behavioral sensitization is dependent on the environmental context and conditioning variables. Animals injected with cocaine in the same environment are more reactive to a subsequent dose compared to animals similarly injected with cocaine in a different environment. A series of studies has indicated that the occurrence of cocaine-induced hyperactivity itself is necessary for the sensitization effect to occur. If the activating effects of cocaine are blocked with neuroleptics or GABA agonists, behavioral sensitization does not occur. Moreover, the degree of similarity of the initial pretreatment environment and the subsequent test environment also determines the degree of behavioral sensitization to cocaine. Taken together, these data suggest an important role for conditioning variables in cocaine-induced behavioral sensitization.

At higher doses, cocaine produces another type of sensitization that is very much related to kindling. Repeated doses of cocaine, which are initially subconvulsive, will eventually produce seizures. With the pure local anesthetic lidocaine, we have documented that this effect is not related to changes in blood levels of the drug and appears to be a true "pharmacological kindling" effect rather than one associated with alterations in pharmacokinetics.

Thus, it appears that chronic cocaine use is associated with symptoms attributable to two major properties of cocaine: 1) the psychomotor stimulant properties thought to be mediated by potentiation of catecholaminergic mechanisms which are involved in behavioral sensitization; and 2) the local anesthetic effects of the drug, which are involved in kindling of seizures. We have found that the anticonvulsant carbamazepine is able to block the development of pharmacological kindling induced either by lidocaine or cocaine. However, once full-blown seizures are manifest, carbamazepine is without effect on lidocaine seizures and may actually enhance lethality during cocaine seizures. These data, taken in conjunction with the finding that carbamazepine does not block the developmental phase of electrically kindled seizures following amygdala stimulation, but is quite potent on completed kindled seizures, indicate that different types of kindled seizures are differentially responsive according to the stage of kindled development. They also secondarily imply that different neurochemical mechanisms may be involved in different stages of the kindling process which had previously been thought to be a unitary phenomenon.

It is also of particular interest in relationship to the recent great public health interest in cocaine that we found that while animals were well able to tolerate repeated induction of lidocaine-induced seizures, this was not the case following cocaine seizures. In several series of animals given repeated administration of the same dose of cocaine, all have died following the first or second cocaine seizure in contrast to the excellent tolerance of repeated local anesthetic-induced seizures induced by lidocaine. These data suggest that the additional psychomotor stimulant properties of cocaine, interacting with the local anesthetic-induced seizure mechanism, markedly increase cocaine-induced lethality.

Clinical data with the non-psychomotor-stimulant local anesthetic procaine (reviewed above) also suggest that many of the alterations in sensory and cognitive processes reported following cocaine in humans can also be attributed to its local anesthetic rather than its psychomotor stimulant properties. Thus, as doses of cocaine are increased in order to attempt to achieve the euphoric effects of cocaine (presumably based on potentiation of catecholaminergic mechanisms), acute and chronic effects of cocaine based on its local anesthetic mechanisms may increasingly come into play. For example, Dr. T.W. Uhde and collaborators have observed many patients who develop panic attacks following repeated self-administration of cocaine; panic attacks may then occur spontaneously. We suggest that this process may follow a kindling-like mechanism based on the local anesthetic properties of cocaine, just as a literal kindling mechanism can occur and account for the late development of seizures following repeated doses in some cocaine users. Taken together, data from the sensitization and kindling perspective indicate additional reasons for the dangerousness of cocaine, in addition to its potential compulsive self-administration -- behavioral and convulsive toxicities may increasingly develop over time and even with repetition of the same dose.

A series of studies conducted in collaboration with Drs. S.R.B. Weiss and A. Pert have revealed that intracerebroventricular administration of corticotropin-releasing hormone (CRH) produces the late onset of seizures (approximately 4 to 8 hours post injection) that are virtually indistinguishable from those produced by kindling of the amygdala. However, these seizures are not reproduced by local administration of CRH into the amygdala, hippocampus, thalamus, hypothalamus, periaqueductal grey, or endopyriform nucleus of the perirhinal cortex. CRF seizures are also not inhibited by small and large lesions of the amygdala and hippocampus. Repeated administration of CRH leads to tolerance to its ictal effects; nonetheless, the experience of CRH seizures facilitates subsequent electrical kindling of the amygdala. Moreover, prior amygdala kindling attenuates CRH seizures but increases the behavioral aggression associated with CRH infusions. These data suggest interesting and complex interactions of an endogenous neurohormone which, in high doses of 50-100 μg i.c.v., can induce profound behavioral effects and seizures in addition to its endocrine effects.

Unit on Behavioral Pharmacology, (Agu Pert, Ph.D., Chief)

In collaboration with Dr. P. Clarke, Dr. A. Pert has studied the behavioral, biochemical, and electrophysiological effects of another widely used and abused drug, nicotine. Nicotine receptors have recently been demonstrated in brain using [^{3}H]nicotine; the binding sites are stereospecific, saturable, and reversible. They are of high affinity and selectively displaced by nicotine agonists, including acetylcholine. Audiographic analysis reveals heavy nicotine labeling in a variety of thalamic nuclei, as well as in the cerebral cortex and substantia nigra. Thus, the distribution of binding is very different from that produced by alpha-bungarotoxin, previously used as a nicotinic ligand. Lesion studies have indicated that nicotine receptors in the substantia nigra are located on the perikarya of dopamine-containing neurons in the zona compacta. The likely source of this innervation is the parabrachial region situated in and around the tegmental pedunculopontine nucleus.

Further anatomical and electrophysiological studies were conducted to define this cholinergic pathway. Unilateral injections of fluorescent retrograde label-fast blue into the zona compacta labeled a diffuse population of neurons in the parabrachial region, a substantial proportion of which possessed CAT-immunoreactivity. Unilateral injections of kainic acid into the parabrachial region produced an immediate dose-dependent excitation of dopamine neurons in the zona compacta. Intravenous injections of mecalamine prevented the kainic acid-induced excitation, revealing the nicotinic characteristics of this effect. These results suggest the cholinergic neurons in and around the pedunculopontine nucleus provide an excitatory input to dopaminergic neurons of the substantia nigra.

Nicotine exerts potent effects on locomotor activity. These effects appear to be mediated through the ventral tegmental region (A10) by direct activation of mesolimbic dopaminergic neurons. The depressant actions of nicotine appear to be mediated through the nucleus accumbens, anterio-ventral and ventrolateral thalamus. It is postulated that the reinforcing and euphorogenic properties of nicotine are related to its ability to enhance the activity of mesolimbic dopamine systems.

Phencyclidine (PCP) is a powerful psychotomimetic substance of considerable import because of the similarity of many of its effects in man to schizophreniform syndromes. A series of studies now document that the behavioral effects of PCP in animals are unlikely to involve dopaminergic mechanisms, a finding of considerable import in relationship to non-dopaminergic theories of psychotic behavior. While reserpine antagonizes methylphenidate-induced hyperactivity and alpha-methyl-para-tyrosine antagonizes that induced by amphetamine, neither pretreatment is effective in blocking PCP-induced hyperactivity. Moreover, 6-hydroxydopamine lesions of the nucleus accumbens which attenuate amphetamine-induced locomotor activity do not block that induced by PCP. In addition, unilateral injections of PCP into the striatum do not produce a symmetry in animals administered amphetamine. Amphetamine-induced rotational behavior is accompanied by significant lateralized alterations in dopamine and DOPAC; again, PCP does not share this effect.

In contrast to striatal injections, unilateral injection of PCP into the nigra produces marked rotational behavior. Knife cuts rostral to the nigra were ineffective in altering rotational behavior, while cuts caudal to the nigra decreased such behavior significantly. These findings suggest that the activity of PCP in the substantia nigra activates pathways which project caudally and are independent of dopaminergic mechanisms.

In collaboration with Dr. N. Ostrowski, the time-course and dose response characteristics of [^{3}H]-cyclofoxy were determined in awake-behavior of rats with autoradiographic techniques. Following intravenous injections of this recently synthesized opiate receptor antagonist, selective accumulation is noted in brain regions that are dense in opiate receptors and little accumulation is noted in areas deficient in opiate receptors. These data support the feasibility of using cyclofoxy as an *in vivo* label for opiate receptors in living animals and strengthens the idea that (^{18}F)-labeled cyclofoxy will be a valuable tool for visualizing opiate receptors using PET procedures in man.

In vitro autoradiography has indicated that adult male hamsters have dense opiate receptors in hypothalamic regions that are known to regulate neuroendocrine and behavioral aspects of reproductive behavior. A region of particularly dense binding was discovered dorsal to the suprachiasmatic nucleus in male hamsters, an area which could mediate opiate effects on photoperiod-induced alterations of reproductive functioning. Circadian-linked fluctuations in the density of opiate receptors were discovered in cycling female hamsters and shown to be related to differences in opiate receptor number rather than affinity. These data suggest possible sites of opiate regulation of reproductive function in hamsters.

In vivo autoradiographic techniques were employed to test the possibility that mating behavior in male hamsters may function as a physiological stimulus to release of opiate peptides in brain. Mated male rats show reduction in the density of [^{3}H]-cyclofoxy binding when the ligand is delivered during a mating session. These data suggest that the ejaculatory response in male hamsters leads to a release of endogenous opiate peptides in brain that compete for occupancy with the [^{3}H]-ligand. Thus, these data provide cogent evidence for regionally specific release of endogenous opiate peptide systems in brain by the physiological stimulus of ejaculation during mating behavior.

In vivo autoradiographic procedures have also been used to assess the functional activity of endogenous opiate systems following electrical stimulation of the arcuate nucleus, the origin of the beta-endorphin system. This stimulation produces profound naloxone-reversible analgesia which was accompanied by decreases in [^{3}H]-diprenorphane binding in areas of brain receiving input from beta-endorphin pathways. Repeated stimulation of the amygdala produces kindled seizures which were also accompanied by decreased binding of [^{3}H]-diprenorphane in many forebrain areas, especially the nucleus accumbens. These data suggest that exogenous stimulation of endogenous opiate pathways as well as their physiological activation during ejaculatory behavior may be associated with release of opiate peptides in brain.

Unit on Neurochemistry, (Paul Marangos, Ph.D., Chief)

The Unit on Neurochemistry focuses on the function of brain proteins such as neuron specific enolase (NSE) and the preclinical characterization of adenosine, benzodiazepine, and calcium channel binding sites. There has also been an increasing focus on the clinical application of findings with NSE.

It is now widely accepted that elevated serum NSE levels are of prognostic and diagnostic import in both oat cell lung cancer and in pediatric neuroblastoma patients. Findings have been extended this year to show that serum NSE levels are superior to other existing methods in predicting relapse in lung cancer patients, a finding that may affect survival time, since this allows earlier resumption of chemotherapy. The development of a kit in collaboration with Hoffmann-La Roche for measuring NSE by enzyme immunoassay has proceeded to the point of FDA submission. This will allow wider availability of this assay and represents another example of a basic research strategy, developed almost entirely at the NIMH, that has resulted in a clinically useful application.

Work with NSE has also proceeded on a molecular biological approach. A full-length cDNA probe for NSE has been obtained in collaboration with Drs. Ginns and Martin. Using sequence data from pure human NSE and non-neuronal enolase (NNE), Dr. Ginns has prepared several cDNA probes for each protein. The mRNA for NSE is now being localized in developing brain and the gene being mapped on human chromosomes. These studies may provide insight regarding neuron specific gene expression as, at the time of synaptogenesis, NNE switches to NSE; available molecular biological tools can now begin to characterize the nature of this mechanism.

The Unit also focuses on characterization of adenosine receptor binding and uptake sites. A new ligand for adenosine receptor uptake site (dipyridamole [DPR]) has been labeled with high specific activity (110 c/mmol) with tritium. Using this unique probe ([^{3}H]DPR), it has been found that 3-4-fold more adenosine uptake sites are labeled than with previously used ligands such as nitrobenzylthioinosine (NBI). This finding suggests that multiple populations of adenosine uptake sites may be present in brain and raises the possibility of developing selective pharmacological agents for these different sites. Dr. J. Deckert has mapped the differential distribution of adenosine receptor binding sites and uptake sites autoradiographically. Dr. P. Morgan has measured adenosine uptake directly into synaptoneurosomes, further documenting differences between NBI and dipyridamole, which complement the binding studies. In these studies, Dr. Morgan has shown that NBI inhibits adenosine uptake in a biphasic manner. This directly supports binding data showing that [^{3}H]DPR is only partially inhibited by NBI. With the development of specific ligand probes for both the adenosine receptor and uptake sites, the physiological role of adenosine receptor systems (which have important effects on sedation, seizures, hypertension, and possibly anxiety) can be systematically explored. Additionally, work is proceeding on purifying the adenosine receptor and the uptake site. Using human erythrocytes, adenosine uptake sites have been photo-labeled and solubilized and resolved on ion exchange columns. Once sufficient purity is achieved, a cDNA probe will be developed in order to study possible molecular mechanisms of receptor modulation.

The relationship between calcium antagonists and adenosine has also been further explored with results showing that the dihydropyridine calcium antagonists and the agonist (BAY-K-8644) are potent inhibitors of binding to the adenosine receptor and uptake site. The hypothesis being explored is that the major presynaptic effects of adenosine (inhibition of neurotransmitter release) are mediated by calcium channels.

Both "central- and peripheral-type" benzodiazepine receptors, as well as adenosine receptors, have been studied across several animal models. Dr. E. Tamborska has found that cerebellar adenosine receptors are increased in Maudsley reactive rats while benzodiazepine receptors remain unchanged. In collaboration with Dr. T. Insel of the LCS, autoradiographic receptor mapping has revealed that the adenosine receptor increases in the Maudsley reactive animals are selective to the molecular layer of the cerebellum. These findings are also of interest in relation to previous findings from this laboratory that chronic stress can upregulate brain adenosine receptors.

Studies have continued concerning the relationship of carbamazepine action to the adenosine system. K. Smith has shown that carbamazepine inhibits agonist binding with a five-fold greater potency at 0° than at 30°. This is characteristic of an antagonist and further suggests paradoxical antagonist effects of carbamazepine at adenosine receptors. An antagonist profile of binding was also observed with several derivatives of carbamazepine, including the clinically active -10,11-epoxide.

Studies are now in progress attempting to further elucidate the effect of carbamazepine on adenosine- and other ligand-stimulated cAMP levels.

Work in this Unit was instrumental in the findings that the anticonvulsant effects of carbamazepine were likely mediated through the "peripheral-type" benzodiazepine receptor, as revealed by both chemical studies of displacement of $[^3H]$-Ro5-4864 binding and the physiological studies reviewed above. Dr. P. Morgan has, in addition, now shown that Ro5-4864 specifically binds to calmodulin. These studies may prove to be important in relation to the recently reported association of "peripheral-type" benzodiazepine receptors and calcium mobilization mechanisms.

Dr. Jitendra Patel has focused his studies on the modulation of neurotransmitter function, particularly involving second messenger systems and protein phosphorylation. He has recently found that a phosphoprotein, S100 modulated phosphoprotein (SMP) is a specific substrate for protein kinase C in intact cells and that phosphorylation of SMP can be induced by phorbol esters (activators of protein kinase C). This activation may also be involved in the process of neurotransmitter and hormone release, as revealed in the studies of pituitary tumor cells. SMP has now been purified using high-pressure liquid chromatography and will lead to the amino acid sequencing as well as the development of antibodies to it.

Dr. Patel has previously found that activation of protein kinase C by phorbol esters causes desensitization of isoproterenol-stimulated adenylate cyclase activity. Thus, it appears that protein kinase C-mediated phosphorylation enhances the uncoupling and translocation of the beta-receptor. Recent work has confirmed that the activation of protein kinase C is associated with its translocation to membrane from cytosol. Concanavin A will block this translocation of protein kinase C and also abolish the effect of phorbol ester on beta-receptor sensitivity.

In related work in collaboration with Dr. Rebois, NINCDS, the molecular mechanisms of gonadotropin-responsive adenylate cyclase have been studied in Leydig cells. Treatment of these cells with phorbol esters causes desensitization of gonadotropin-responsive adenylate cyclase. This process was indistinguishable from that produced by the direct agonist treatment. Thus, current direct evidence suggests that phorbol ester and agonist-induced desensitization may occur via protein kinase C-mediated phosphorylation of the receptor or a receptor-linked protein. Identification of specific phosphoproteins involved in this process is presently in progress.

In collaboration with Dr. R. Fine of the NCI, Dr. Patel has elucidated an important and novel role for protein phosphorylation in anti-cancer drug resistance. Cell resistance appears to involve a 20 kilodalton phosphoprotein that is virtually absent in similar cells which are sensitive to anti-tumor drugs. Activation of protein kinase C in cells sensitive to anti-tumor drugs causes enhanced efflux of the antitumor agent and causes sensitive cells to escape the toxic effects of these drugs. Thus, the cells behave like resistant cells following the activation of protein kinase C. The 20 kilodalton protein is one of the major proteins that becomes phosphorylated following activation of protein kinase C. Moreover, in resistant cells, the phosphorylation of the 20K protein was significantly greater than in sensitive cells. The protein kinase C activity in these cells was elevated 6-fold compared to the sensitive cells. These observations suggest that tumor cells become resistant by effluxing the anti-tumor drug at a greater rate. Efflux of the drug appears to be under the control of protein kinase C.

Phosphoprotein phosphatases are a class of enzymes involved in dephosphorylation of proteins. Calcineurin is a phosphatase whose activity has been shown to be regulated by calcium-calmodulin. Using purified preparations of protein kinase C and calcineurin, Dr. Patel has recently demonstrated that calcineurin is phosphorylated by protein kinase C and that this phosphorylation modulates the interaction of the enzyme with calmodulin. This finding suggests that the phosphorylation states of some proteins may be regulated by dephosphorylation as well as phosphorylation.

In collaboration with Dr. D. Jacobowitz of the Laboratory of Clinical Science, Dr. Patel has identified a novel 94K substrate of cyclic-AMP-dependent protein kinase that is localized to various regions of caudate, habenula, and the interpeduncular nucleus. These data and others suggest that the 94K protein may reside in cholinergic neurons. Purification of this protein is currently in progress.

Dr. Patel, in collaboration with Dr. E. Klein, has discovered that chronic treatment of rats for three weeks with lithium carbonate produces marked increases in the phosphorylation of a 64 kilodalton protein. The effect was observed in caudate, putamen, hippocampus, cortex, and cerebellum, and was sub-cellularly localized to a membrane fraction of the preparation. It is possible that this 64K phosphoprotein may represent the beta-subunit of calcium-calmodulin-dependent protein kinase or a co-migrating protein. Further work with chronic exposure (48 hours) of cultured anterior pituitary tumor cells to lithium has shown profound changes in the second messenger systems of the cell. Specifically, lithium caused an increase in calcium dependent protein phosphorylation in general and specifically in protein kinase C activity. Direct exposure of purified protein kinase C to lithium had no effect. The physiological significance of this upregulation of protein kinase C is presently being evaluated and suggests new avenues for considering the mechanisms of action of lithium carbonate.

ANNUAL REPORT OF THE CLINICAL NEUROGENETICS BRANCH

National Institutes of Mental Health
October 1, 1985 - September 30, 1986
Elliot S. Gershon, M.D., Chief

The Clinical Neurogenetics Branch does clinical and pertinent basic investigations in genetics of major psychiatric disorders. The investigative thrust of this branch is to combine the most advanced understandings and methodologies in genetics, including molecular genetics and population genetics, with careful biological and diagnostic investigation of specific psychiatric and neurologic disorders. The areas of investigation may be subsumed under human gene mapping, peptides and proteins important in the CNS, disease related physiologic events, and epidemiologic-genetic diagnostic studies.

1. Human gene mapping. The major laboratory methods used are DNA restriction fragment length polymorphisms (RFLPs), and 2-dimensional protein electrophoresis (2DE). New RFLPs were identified for vasoactive intestinal peptide (VIP), and adenosine deaminase (ADA). Comparison of the variant neuropeptide Y alleles (discovered last year) in normals and patients with schizophrenia and bipolar affective disorder did not show different allele frequencies. Linkage analysis of the neuropeptide Y region in pedigrees demonstrated that it is not linked to inheritance of manic-depressive illness.

We are also undertaking to assemble a collection of cells from well-diagnosed pedigrees with manic-depressive illness and schizophrenia. With the proliferation of laboratories capable of doing RFLP linkage studies, the absence of such a resource has put constraints on progress in this area.

2. Peptides and proteins important in the CNS. Advanced methods of silver staining of proteins and of nucleic acids on gels or blots, developed by Dr. Merril, offer increased sensitivity of detection over previously used stains. These methods have been further advanced by the discovery of the importance of basic and sulfur-containing amino acids for the staining reaction, leading to the possibility of more sensitive protein detection methods by coupling proteins on the gel or blot to appropriately constructed polymers prior to silver staining.

Neuropeptides continue to be studied in cerebrospinal fluid (CSF) using radioimmunoassay, as well. The consistency of individual differences, and the consistent correlations between concentrations of several of the neuropeptides studied, suggest genetic control over CSF neuropeptides as a system. Twin studies of these peptides, and attempts to develop electrophoretic methods for CSF peptides, are ongoing.

The muscarinic acetylcholine receptor from bovine brain has been purified to near homogeneity. The specificity of pirenzepine, a ligand which initially defined a muscarinic receptor subtype, was demonstrated to be lost after solubilization of this receptor protein. An endogenous factor which acts as a muscarinic agonist, but is not acetylcholine, has been isolated from brain; further characterization of this substance is proceeding.

3. Disease related physiologic events. Abnormal disease-related CSF proteins have been discovered in this branch, using silver staining on 2DE gels, for Creutzfeld-Jacob disease, herpes encephalitis, schizophrenia, Guillain-Barre, and Parkinson's disease. Further studies demonstrating the specificity of these findings have been carried out this year, and attempts to isolate the disease-specific spinal fluid proteins are ongoing. This may lead to advances in understanding of the disease process, genetics, and diagnosis, in these diseases.

In bipolar illness, we have studied cholinergic induction of rapid eye movement sleep, and density and regulation of beta-adrenergic receptors on peripheral cells. Although both of these phenomena had been reported to distinguish manic-depressives from normals, we found only a weakly increased sensitivity to the cholinergic stimulus and no difference in the beta receptors, suggesting that neither of these phenomena would prove useful in genetic studies of the illness.

In schizophrenia, computed tomographic studies of familial schizophrenia did not support the reported reduced cortical size and skull circumference reported by others. We found no association of anueploidy or fragile sites on chromosomes with schizophrenia.

4. Genetic epidemiology. In the affective disorders, we have identified a birth cohort effect on the prevalence of mania, compatible with an ongoing increase in a spectrum of affective and related disorders, including mania, depression and suicide. A family study of rapidly cycling bipolar patients revealed that relatives of rapid cyclers had a lower risk of affective illness than did relatives of other bipolars. One implication is that non-genetic factors may contribute more frequently to the rapid-cycling form of this disorder. Family studies of schizophrenia, bulimia, and a high-risk study of bipolar affective illness, are ongoing.

This brief survey demonstrates the extent of interleaved basic and clinical investigations in the CNG, and the degree to which advances in genetics are applied to the problem of identifying specific markers of inherited vulnerability in the major psychiatric disorders.

Patent Awarded

Merril, C.R.
U.S. Patent 4,555,490: A rapid photo-reduction silver stain for the visualization of proteins and nucleic acids, March 1986.

Awards

Ripley Award Lecture, University of Washington, 1985. Dr. Elliot Gershon

SECTION ON CLINICAL GENETICS

Elliot S. Gershon, M.D.

The Section on Clinical Genetics has as its goals: identification of biological and psychological factors that are genetically transmitted in major psychiatric disorders, investigation of genes active in the central nervous system, and pharmacologic and pharmacogeneic studies of psychoactive agents in psychiatric patients and in normal controls.

Several lines of investigation are pertinent to genetics and clinical neuroscience in general, rather than to specific diagnostic entities.

We continue to study genetic polymorphism in genes for substances important for CNS function, and through linkage studies in pedigrees and sib-pairs, to test whether there is any abnormality in these genes in major psychiatric disorders.

Restriction fragment length polymorphisms (RFLPs) were identified for the structural genes of neuropeptide Y (NPY), vasoactive intestinal peptide (VIP) and adenosine deaminase (ADA) (Dr. Detera-Wadleigh). Comparison of variant neuropeptide Y allele frequencies in both normal individuals and patients, that is, schizophrenics and manic-depressives show no significant difference. The linkage relationship between NPY and a putative major locus for manic-depressive illness was examined in two pedigrees. The lod scores obtained under various assumed modes of inheritance suggest absence of linkage. Genes for two other substances have been isolated: human calmodulin cDNA has been isolated and partially sequenced. A neurotensin specific cDNA clone was isolated from a rat brain lambda-gt11 library.

The muscarinic receptor from bovine brain was purified to near homogeneity (Dr. Baumgold.) After solubilization with digitonin, the preferential binding of pirenzipine to cortex as opposed to medulla/pons receptors is lost. This implies that the M1/M2 receptor subtyping, which was originally defined by this preferential binding, may not be inherent in the receptor ligand-binding protein. The calcium channel blocking agent verapamil was found to affect the binding characteristics of muscarinic receptors. An endogenous factor, not acetylcholine, which acts as a muscarinic agonist, has been isolated from brain, and further characterization of this substance is proceeding.

We have continued to study receptor-like characteristics on peripheral cells (Dr. Berrettini.) We have been unable to confirm our preliminary indications that there exist somatostatin (SRIF) and vasoactive intestinal peptide (VIP) receptors on skin fibroblasts.

Clinical investigations attempt to identify genetic markers and biologic vulnerability factors in major psychiatric disorders.

The clinical basis for these investigations is systematic diagnostic study of patients, normals, and relatives of each, along with collection of physiologic information and biologic specimens from them. These specimens include most notably the cell collections that have been developed for schizophrenic and manic-depressive pedigrees, with appropriate controls, and cerebrospinal fluid (CSF) which has been studied in a large series of drug-free patients and controls. Family diagnostic studies of schizophrenia, bulimia, and of rapid-cycling manic-depressive illness are currently in progress. A high-risk study of offspring of bipolar patients is ongoing, where people are studied at an age (15-21) when mood disorder is most likely to develop.

A major bottleneck to clinical genetic investigations exists at this time, because of the lack of adequate cellular collections of pedigrees and affected sib-pairs in the psychiatric disorders, including schizophrenia, manic-depressive illness, and Alzheimer's disease. We are building such a cellular collection, in which each individual is systematically assessed psychiatrically (Drs. Delisi, Berrettini.) This is a major clinical investigative effort, and we estimate it will be two to three years before the collection is large enough to serve as a national resource. At this time, we have in culture twenty affected sib-pairs with schizophrenia, four manic-depressive pedigrees, and two schizophrenia pedigrees. Mathematical analysis (Dr. Goldin) has demonstrated that with a realistically achievable sized collection, linkage is detectable even in the presence of a considerable genetic heterogeneity.

The beta-adrenergic receptor exists on skin fibroblasts and transformed lymphocytes. No differences were found between manic-depressives and controls in the baseline sensitivity or desensitization of these receptors, contrary to previously published reports (Dr. Berrettini.)

The CSF offers important investigative opportunities, despite the difficulty in obtaining specimens. The stability of individual differences in concentrations of 12 neuropeptides, and their intercorrelations imply that these differences may be genetic and of physiologic importance (Dr. Berrettini). Studies of CSF neuropeptides in psychiatric patients and in normal controls found that neuropeptide Y, growth hormone releasing factor, and atrial naturietic factor were not significatly different between several diagnostic categories of patient and controls.

Pharmacologic challenge studies have attempted to probe cholinergic and serotonergic function in affective disorders (Drs. Berrettini, Nurnberger.) The muscarinic cholinergic agonist arecoline was used to induce rapid eye movement (REM) in sleeping subjects. Our replication study is completed, and does not show differences between 18 euthymic bipolars and 18 controls. The serotonin precursor tryptophan did not show increased sensitivity in bipolars as compared with controls, when induced cortisol secretion was the dependent variable.

Cytogenetic studies of schizophrenia revealed no increased aneuploidy or fragile-X frequency, in comparison with controls (Dr. Delisi.)

Viral studies in schizophrenia yielded no association of illness with herpes, Epstein-Barr, or HLTV antibodies. Treatment of patients who had elevated Epstein-Barr antibody titers with acyclovir did not produce beneficial effects (Dr. Delisi.)

CT studies of familial schizophrenia did not replicate the reduced cortical size and skull circumference reported by others (Dr. Delisi.)

Epidemiologic genetic studies continue to yield important results. In the affective disorders, we have identified a birth cohort effect on the prevalence of mania, such that in more recently born cohorts there are higher rates than in earlier born cohorts (Dr. Gershon.) These data are compatible with an ongoing increase in a spectrum of affective and related disorders, including mania, depression and suicide. A study of adolescent and young adult well offspring of bipolar patients, ages 15-21, showed these young people to have more psychological disinhibition than controls (Dr. Nurnberger.) This may constitute a behavioral marker of vulnerability; follow-up of these persons continues. A family study of rapidly cycling bipolar patients revealed that relatives of rapid cyclers had a lower risk of affective illness than did relatives of other bipolars. One implication is that non-genetic factors may contribute more frequently to the rapid-cycling form of this disorder.

SECTION ON BIOCHEMICAL GENETICS

Carl R. Merril, M.D., Chief

This Section conducts research on genes and gene products in physiological and pathophysiological states. The Section introduced the use of silver staining for the detection of proteins and nucleic acids separated by polyacrylamide gel electrophoresis in 1979 and has recently developed more rapid silver staining protocols and stains which can detect proteins and nucleic acids separated on membranes such cellulose nitrate. The stain introduced in 1979 required three hours to perform while current staining procedures with the newly developed protocols require under half an hour. These new stains also provide a rapid quantitative method of analyzing samples, by staining samples spotted on nitrocellulose membranes, for proteins and nucleic acids in the nanogram range.

Studies concerning the mechanism of silver staining have revealed the importance of the basic and sulfur containing amino acids for protein detection and the purine bases for nucleic acid detection. This discovery of the importance of the basic and sulfur containing amino acids offers the opportunity to use basic or sulfur containing homopolymers to enhance the staining of proteins separated by electrophoresis. By coupling these homopolymers to the proteins prior to staining the number of stain reactive groups would be increased, with the potential for an increase in the sensitivity of protein detection. Such enhanced protein detection could provide for an increased ability to visualize the large number of trace proteins that must be present but still cannot be detected by our current stains, which are capable of detecting a 0.1ng of protein. Surveys of mRNA in mammalian brain tissues have indicated that as many as 70,000 proteins may be synthesised, however using the best separation and detection methods only 3000 proteins can be visualized. The development of more sensitive protein detection methods will provide an opportunity to study additional proteins in pathophysiology processes that are of importance to the Institute.

The Section is also developing automated methods for the analysis of the complex electrophoretic patterns that are obtained from human body fluids, such as serum and spinal fluid, and cellular lysates. Computer programs are being developed to provide for the rapid comparison of groups of gels from clinical studies to provide for the identification and scoring of polymorphic proteins and other quantitative and qualitative protein alterations.

Analysis of spinal fluid proteins separated by electrophorsis and detected by silver staining has led to the discovery of five abnormal, disease associated proteins. One (molecular weight of 25Kd) of these proteins appears to be specific for Parkinson's disease, two others (26 and 29Kd) are specific for Creutzfeldt-Jakob disease and herpes encephalitis, and the two additional abnormal proteins (40Kd) are found in Schizophrenia, Creutzfeldt-Jakob, herpes encephalitis, Guillain-Barre and Parkinsons disease. The 26 and 20Kd proteins that are specific for Creutzfeldt-Jakob disease and herpes encephalitis have been shown to be absent in other causes of dementia (AIDS-associated dementia, multiinfarct dementia, Alzheimer's disease, Huntington's disease, Parkinson's dementia of Guam) and other infectious disorders of the CNS (including tuberculous, syphilitic meningitis, measles encephalitis, cytomegalovirus infection, varicella rubella and three togaviruses). The specificity of these two 26 and 20Kd proteins for Creutzfeldt-Jakob, and herpes encephalitis illustrates their potential as diagnostic markers for these diseases.

The Section is currently isolating the disease specific spinal fluid proteins. These proteins will be partially sequenced and DNA and antibody probes will be constructed. The DNA probes will allow us to ask whether these disease specific proteins are of human or exogenous, possibly viral, origin. The antibodies generated for these proteins will permit rapid screening of patients and they may provide new diagnostic tools for Schizophrenia, Creutzfeldt-Jakob, herpes encephalitis, Guillain-Barre and Parkinsons disease. The development of these new diagnostic tools and their potential for furthering our understanding of diseases affecting the central nervous system are important for advancing the goals of our Institute.

Charge variant, polymorphic proteins that have been identified by the section are being used in genetic linkage studies involving schizophrenic and families with affective disorders. The discovery of a genetic linkage with any of the polymorphic proteins would provide additional diagnostic aids for these diseases and would permit the localization of the disease gene(s). The localization of these disease gene(s) would provide the institute with the potential of determining the genetic basis of these diseases, a reliable method of diagnosis, and a means to further our understanding of these diseases.

The Section is currently developing techniques to screen for single base substitutions in the mitochondrial genome. Numerous studies concerning the mitochondrial genome have indicated that the mutation rate of this closed circular 16.5 Kb circular DNA molecule is relatively high when compared to the nuclear genome. Grossman, Watson and Vinograd, have found an average of 5 ribonucleotides per strand in animal mtDNA suggesting that the editing function of the mtDNA replication complex may be inefficient or lacking. Furthermore, mitochondria lack the capability of excising or repairing thymidine dimers and there is evidence that mitochondria also lack the ability to repair other types of DNA damage. Mitochondrial DNA also has a higher turnover rate than the nuclear DNA providing more rounds of replication during which errors could be generated.

In a survey by Monnat,and Loeb concerning nucleotide sequence preservation of human white blood cell mitochondrial DNA they found only 1 bp variation in 13,405bp examined from one individual. However, they did not examine postmitotic tissue. Mitochondrial DNA from postmitotic tissues such as the neurons in the CNS might serve as a good indicator of genetic insults and catastrophic events which may occur as disease and aging processes. Particular regions such as the hypervariable regions near the origin of replication might be particularly useful in this endeavour as it is a non-coding region that shows considerable variation in intra-individual comparisons.

These intra-individual variations in the hypervariable regions are being studied in a collaborative project with the FBI Forensic Science Research Group to determine whether the intra-individual nucleotide variations are sufficient to establish individuality in forensic studies. Oligonucleotide primers have been synthesized to facilitate direct seqencing of the hypervariable regions of mitochondrial genome.

Annual Report of the Clinical Psychobiology Branch
National Institute of Mental Health
Thomas A. Wehr, M.D., Chief
October 1, 1985 through September 30, 1986

The principle aim of the Clinical Psychobiology Branch research program is to investigate the causes of depression and mania and to develop new approaches to the treatment of these disorders. Affective disorder is a recurrent illness characterized by spontaneous relapses and remissions. Sometimes the illness takes a circular course with mania and depression alternating in a quaisi-periodic manner. The circular, cyclic forms of the illness are particularly suitable for clinical investigation because the predictable recurrence of episodes makes it possible to conduct prospective longitudinal studies of the processes that leads to depression and mania. In some of these cases the cycles of the illness are synchronized with the seasons of the year. Our investigations of these seasonal forms of affective illness have been particularly fruitful.

We found that there are two types of seasonal affective disorders. In one type, patients regularly become depressed in the fall and winter and hypomanic in the spring and summer. In a second, opposite type, patients regularly become depressed in the spring and summer and hypomanic in the fall and winter. In the first type we hypothesized that seasonal changes in natural light triggered the seasonally recurring episodes, and that the course of the illness might be modified by exposing patients to bright artificial light. We found that phototherapy is an effective treatment for fall-winter depression, and several other groups in the United States and Europe have confirmed our results. In the second type of seasonal affective disorder our preliminary evidence indicates that seasonal changes in environmental temperature may trigger the seasonally recurring affective episodes and that modifications of environmental temperature may be an effective treatment for spring-summer depression. The effects of seasonal changes in environmental temperature on clinical state may be mediated by abnormal responses of the hypothalamic-pituitary-thyroid axis, which is impaired in a large number of these patients. We feel that these findings are rather remarkable in that they show that manic-depressive illness is very sensitive to specific, physical conditions in the environment. We and others have also shown that the illness is quite sensitive to environmental and behavioral factors that affect sleep.

Hibernation, estivation and other seasonal changes in animal physiology and behavior seem to be suitable models for the seasonal forms of affective illness. These animal behaviors are adaptations to seasonally recurring changes in the availability of food and ambient temperture, which challenge the energy economy of the organism. In this regard, it is interesting to note how many symptoms of manic-depressive illness involve changes in the acquisition, storage and expenditure of energy. These symptoms included changes in appetite, eating, weight, activity and rest, and exposure to and withdrawal from the environment. Sleep, which changes dramatically in mania and depression, may have an energy conserving function analogous to torpor, hibernation and estivation. Fundamentally depression may represent an exaggeration of mechanisms directed at energy conservation, and mania an exaggeration of mechanisms directed at energy expenditure (which often serves energy acquisition). Non-seasonal forms of the illness may be equivalent to seasonal rhythms in energy metabolism that have escaped environmental control and become autonomous. In fact, in individual cases, we have observed that antidepressant medications can transform the course of the illness from a seasonal one to a rapid cycling one. Thus according to this mode, depression and manic-depression are illnesses of systems that manage the energy economy of the organism and that adapt to seasonally changes in environment conditions.

The hypothalamus is the locus of the principal elements of such a system. It receives neural input which conveys information about environmental light and temperature. It controls the level of metabolism, partly through the hypothalamic-pituitary-thyroid axis. It has a biological clock that regulates the timing and amount of activity and rest, wakefulness and sleep, and through these behaviors it regulates the organism's exposure to changing conditionsin the environment. It also regulated appetite and weight, and through its control of metabolism, the thyroid axis, behavior, and the autonomic nervous system, it controls body temperature. Nearly all these functions are disturbed in mania and depression, and, nearly all the symptoms of mania and depression can be attributed to changes in these hypothalamic functions.

Our research program has led to three non-pharmacological treatments involving manipulations of light, temperature and sleep. It has also led to specific concepts about the nature of affective illness, and its anatomical locus. In the future, adequate assessment of the functional state of the hypothalamic energy management system will require new facilities and new procedures in which patients' physiological and behavioral responses to environmental challenges involving light, temperature and sleep manipulations can be properly measured.

Overview of the Seasonality Study, 1985-1986

Norman E. Rosenthal, M.D.

In previous years, we have described the syndrome of seasonal affective disorder (SAD), a condition characterized by recurrent fall-winter depressions alternating with non-depressed periods in the spring and summer. We have shown that treatment of SAD patients with bright artificial light is capable of reversing the winter depressive symptoms of SAD. We have also investigated the possible biological abnormalities underlying this condition and have attempted to understand the mechanisms by which phototherapy works. This past year, we have extended our previous studies in several areas. These will be discussed in this section under headings which correspond to the three separate annual reports from this program.

1. Effects of Light Interventions in Seasonal Affective Disorder

Since we first demonstrated that the winter symptoms of SAD could be successfully treated with bright artificial light, we have replicated the finding twice. This finding has subsequently been replicated by several other groups. We have previously investigated several formal properties of phototherapy and reached certain conclusions; for example, that it is not dependent on depriving subjects of sleep, that the morning hours are not critical for effective treatment, and that the antidepressant effect is not photoperiodic in nature (i.e. it does not depend on extending the naturally illuminated period of the day).

During this past year, we have extended our investigations of the formal properties of phototherapy in SAD by means of three separate studies. We have investigated: (1) whether the phototherapeutic effect is mediated via the skin or the eye; (2) whether treatment with two hours of bright light is better if administered in the morning as compared with the middle of the day; and (3) whether bright light has mood elevating effects in normal individuals, who do not present with a complaint of seasonal mood changes. The results of the three studies were as follows:

1. The skin-eye study: In 10 SAD patients we showed that four hours of evening light exposure to the eyes (with skin covered) produced a significantly better antidepressant response than an equivalent amount of light exposure to the skin (while subjects wore dark goggles). A complicating factor in this study was that expectations of the two light conditions, which we measured systematically for the first time this past year, were significantly correlated with the change in mood rating. This leaves open the possibility that expectation of the different light conditions contributed to the outcome of the study. However, the more likely explanation that the difference in results is due to the antidepressant effect being mediated via the eye and not the skin is of both practical and theoretical importance.

2. The morning-evening light study: In 16 SAD patients, we showed that two hours of morning light exposure was equivalent in antidepressant efficacy to two hours of light treatment in the middle of the day. This finding is of practical significance and argues against a current theory that the efficacy of light is predicated on its ability to shift the phase of circadian rhythms.

3. The study of light in normals: We showed in 11 normal subjects that two hours of exposure to bright full-spectrum light in the morning had no significant mood altering effect. Control treatment with dimmer light in another 11 normals was equally ineffective. This study showed that the mood altering effects of light cannot be generalized to all populations, though it does not rule out the possibility that normal subjects selected and treated in a different way and in different lighting conditions might not benefit from enhancement of their lighting environment.

2. Neurobiology of Seasonal Affective Disorder

In earlier years, we have looked for biological markers of SAD without much success. The hypothalamo-pituitary-adrenal axis, which has been found to be overactive in other depressed populations has appeared normal in our studies of patients with SAD. Similarly, we found in a small number of SAD patients that the TRH stimulation test was normal. The only biological abnormality we were able to document in patients with SAD was hypersomnia during the winter months, with a tendency to reduced slow wave sleep.

This past year, we reanalyzed our baseline thyroid values (thyroxine (T_4), tri-iodothyronine (T_3) and TSH) in our SAD patient and compared them with age- and sex-matched controls. While previous analyses had not revealed any differences between SAD patients and controls in baseline thyroid values, a reanalysis with larger numbers of subjects revealed that in 20 SAD patients baseline winter T_4 values were elevated (mean + S.D.= 8.9 ug/dl) as compared with (7.8 ± 1.35 ug/dl) in 20 normal controls ($\overline{P}$<.01). However, there were no differences between groups in T_3 or TSH values. The finding of abnormal thyroid values is in keeping with other suggestions that in SAD there is a change in utilization of energy between summer and winter; for example, there are changes in appetite, food choice, weight and energy levels. These diverse seasonal changes in behaviors associated with energy utilization and in thyroid hormone levels are reminiscent of the seasonal rhythms in energy conservation, which are widespread among lower animals and which have been widely studied in lower animals. It is our hope that we can use the animal literature along with the technology available to measure metabolic functions in humans to explain the clinical symptoms of SAD and their dramatic response to bright environmental light.

In previous years, we pursued a "melatonin hypothesis" of SAD and phototherapy, patterned on studies of seasonal reproductive rhythms in animals (See last year's report #Z01 MH02221-01 CP). Although there was some suggestion that melatonin secretion may be partly responsible for the symptoms of SAD, at least in some patients, the studies did not bear out the idea that melatonin plays a critical role in the evolution of the syndrome of SAD in most cases. One positive finding to emerge from the melatonin studies was the observation that plasma prolactin levels are abnormally elevated in SAD patients (N=10) compared to normals. Challenging these patients with 5-hydroxytryptophan (5-HTP), a melatonin precursor that has been found to induce plasma melatonin elevations in sheep, did not have this effect in 9 out of 10 SAD patients. However, one patient, who was found to hypersecrete melatonin under baseline conditions, did in fact show a rise in melatonin following the 5-HTP challenge. Further neuroendocrine effects of 5-HTP administration, namely increased cortisol and decreased prolactin levels in plasma, were no different in patients and controls.

This past year, we have continued our investigation into biological correlates of SAD and the effects of phototherapy in a number of ways. We also used bright artificial light, the stimulus that has been shown to produce profound antidepressant effects in this population, as a challenge paradigm, and measured its effects on several relevant biological parameters. Thus, we studied a variety of measures in SAD patients both during baseline and chronic light treatment conditions and compared the results with those of age- and sex-matched normal controls. We studied patients on and off light treatment in counterbalanced order so as to avoid an ordering effect. The parameters measured included: sleep, temperatrue and activity patterns, 24-hour plasma hormone profiles (prolactin, growth hormone, melatonin, cortisol and TSH), 24-hour urinary monoamine metabolite patterns, spinal fluid neurotransmitter metabolites, lymphocyte mitogen stimulation patterns, evoked response potentials, and plasma norepinephrine levels both in recumbent and standing positions.

Although not all studies have been fully analyzed at the time of this writing, the following findings have emerged: 1) The P300 component of the evoked response potential differed under on-light and off-light conditions in 7 SAD patients. The amplitude of the response to visual but not to auditory stimuli was enhanced by light treatment. There was significant correlation between amplitude of the P300 and change in Hamilton Depression Rating, (r= 0.85; P<.02). 2) Mitogen stimulation was suppressed on the on-light condition in 8 SAD patients. Some of the other measurements show interesting trends towards either patient-normal or on-light/off-light differences, but we do not as yet have sufficiently large numbers to make meaningful statistical statements. For example, mean (± standard deviation) level of CSF 5-HIAA in 5 SAD patients was 73.0 ± 31.7 pmole/ml, compared to levels of 96.1 ± 33.6 for 62 non-seasonal depressives previously measured in the same laboratory. Similarly, mean (± S.D.) of CSF HVA in the same 5 SAD patients was 123.5 ± 49.7 pmole/ml compared to values of 163.6 ± 76.3 for the same 62 non-seasonal depressives. CSF levels of MHPG, however, were very similar in the two groups. It would be premature to comment on the rest of the data, as sample sizes are still too small and some of the data have not as yet been completely analyzed. We are planning to increase our sample size the coming year.

The abnormally elevated prolactin levels observed in our patients led us to speculate about the possible underlying neurotransmitter abnormalities. Prolactin secretion is influenced by a number of neruotransmitter and hormonal influences. For example, serotonin is known to stimulate prolactin release and dopamine to inhibit it. In order to explore this question further, we started a project, together with Bud Mueller, M.D., investigating whether the antidepressant effects of phototherapy are associated with changes in serotonergic function analgous to those changes observed with other antidepressant modalities. The paradigm involves studying the effects of intravenous infusions of intravenous infusions of the post-synaptic serotonin agonist mCPP on prolactin and cortisol secretion in patients with SAD before phototherapy (when depressed) and after a week of photherapy (when remitted). Along with the SAD patients, we are studying age-, sex-, and weight-matched controls with two mCPP infusions spaced a week apart. This study is ongoing at the time of this writing.

3. Relationship between eating and mood in seasonal affective disorder.

Patients with SAD report marked seasonal changes in eating behavior and weight. During the winter they report overeating (70% of 182 cases), weight gain (74% of 175 cases) and carbohydrate craving (68% of 161 cases). These symptoms can all be viewed as alterations in energy utilization. We have shown that even a week of treatment with bright artificial light tends to reverse these symptoms, the effect being most marked on carbohydrate craving.

This past year, we studied the effects of different types of meals on SAD patients and normal controls. Thus, we compared the effects on mood of isocaloric lunches containing either high levels of protein or high levels of carbohydrate, in a random ordered, cross-over design. We found that the high-carbohydrate meal tended to make both SAD patients (N=16) and age- and sex-matched normal subjects (N=16) feel less tense ($P<.01$), depressed ($P<.02$) and angry ($P<.05$), as measured by the Profile of Mood States (POMS). However, the effects of the different meals on fatigue levels differed between SAD patients and controls. Thus normals reported an increased level of fatigue following the high-carbohydrate meal, a finding that is compatible with earlier studies, whereas SAD patients showed decreased fatigue levels after the high-carbohydrate meal ($P<.05$).

This energizing effect of carbohydrate may explain, at least in psychological terms, why SAD patients crave carbohydrates in the winter. It would be exremely useful to understand the biochemical correlates of the mood states corresponding to these states of mind (i.e. carbohydrate craving and feelings of increased energy following carbohydrate ingestion). One possible explanation is that dietary carbohydrates are modifying these psychological states by their effects on serotonin metabolism. Thus animal studies have shown that dietary carbohydrate increases the ratio of plasma tryptophan to the other large neutral amino acids by eliciting an insulin response. This amino acid ratio appears to be an important determinant of the amount of tryptophan passing into the brain, which in turn appears to be a rate-limiting step in serotonin synthesis. We have collected blood samples at all time points when psychological measures were taken and their analysis may speak to the degree of plausibility of the above theory as an explanation for our observed results.

The strategy we have used this past year is only one of several possible ways of exploring the relationship of the seasonal changes in eating and mood. If the eating changes are viewed as only one aspect of a seasonal rhythm in energy utilization, several possible lines of inquiry can be pursued to investigate this rhythm further. Thus, eating, weight, metabolic rate and the various hormones involved in modulating these factors could all be studied. Seasonal rhythms of energy utilization in lower animal provide useful models for studying SAD patients.

The inadequacy of the seasonal reproductive animal models, which we pursued previously, make this new set of animal models particularly appealing, especially since melatonin does not appear to be so pivotal an intermediary hormone in the rhythms of energy utilization as in the rhythms of reproduction.

Circadian Rhythms in Affective Disorders

David A. Sack, M.D.

Although mania and depression are generally regarded as disorders of mood, changes in sleep and activity are nearly constant features of these syndromes. Longitudinal observations of bipolar patients made in our laboratory have confirmed prior anecdotal reports that switches into mania tend to occur in close association with one or more nights of sleeplessness. Furthermore, experimental manipulation of the timing and duration of the sleep period in depressed patients has been shown to have acute antidepressant effects in both unipolar and bipolar patients, strengthening the relationship between alterations in sleep schedule and changes in mood. The timing and duration of sleep are regulated in part by a circadian oscillator located in the hypothalamus. Disturbances in this oscillator or in its response to time cues in the environment could be responsible for the sleep and activity changes that occur in affective disorders.

Circadian rhythms can be described in terms of their timing (phase), their amplitude (peak to trough) and in their mean level (mesor). The timing of hormones (cortisol, prolactin), neurotransmitter metabolites (MHPG), motor activity, REM sleep and core body temperature appear to be shifted to an abnormally early time in depressed patients. In addition the amplitude of these rhythms in a number appears to be reduced (temperature TSH, melatonin, prolactin).

One problem common to clinical studies of circadian rhythms is that the behavior of a biological oscillator cannot be measured directly in humans; instead, its behavior must be inferred from the measurable rhythms in the physiologic processes that are regulated by it. As we have noted in earlier reports these measurements can be distorted by a variety of factors called masking, including sleep schedules, physical activity, diet, and environmental lighting, that may not affect the timing of the biological clock. Since patients often exhibit insomnia, anorexia and hypoactivity it is possible that patient-normal differences differences in circadian rhythms arise from differences in masking rather that from an alteration in the endogenous pacemaker system. In order to control for differences that might arise from masking we have measured the circadian rhythms in body temperature, cortisol, melatonin, prolactin, TSH and the neurotransmitter metabolites, MHPG and HVA, in patients with affective disorders and normal controls under routine ward conditions and repeated these measurements under conditions where the effects of diet, activity, posture and wakefulness have been held constant. Although much of this data remains to be analyzed, several preliminary findings are particularly noteworthy.

In previous studies that phase of the body temperature rhythm in depressed patients has been reported to be either advanced, more variable or normal and that mean body temperature has usually been found to be increased. In our study, we found that depressed patients had higher core body temperature than did controls at baseline and that phase of the temperature rhythm was more variable than in the controls. Under constant conditions (supine, bedrest, hourly isocaloric feedings, sleep deprivation) the depressed patients continue to exhibit higher core temperature than did controls; in addition there was a four hour phase-advance in the temperature rhythm of the depressed patients compared with the controls. In two manic patients a pattern of elevated body temperature and a phase advance in the temperature rhythm was also observed on both conditions. These findings suggest that the failure of previous studies to find a phase advance in the temperature rhythm may have been due to masking. The persistence of elevated body temperature in the depressed patients during constant conditions indicates that altered body temperature is not likely to be due to behavioral differences between patients and controls.

We have previously reported that the nocturnal rise in TSH was reduced and the rise in TSH in response to sleep deprivation was diminished in patients with rapid-cycling manic-depressive illness. As part of our present have measured TSH in a group of 8 depressed patients and 8 normal controls as part of the larger circadian study. Our preliminary analysis shows that the depressed patients lack a normal circadian variation in TSH but their mean TSH secretion is not different from controls. These data suggest that the possibility that there may be a continuum of hypothalamic-pituitary-thyroid disturbances in depression; a loss of the circadian variation in TSH could represent the mildest abnormality followed by decreases in nocturnal TSH levels and finally manifesting as frank hypothyroidism as occurs in the rapid-cycler group. The nocturnal rise in TSH is affected by environmental and body temperature; higher temperature is associated with a diminished nocturnal rise in TSH. It is possible that the elevated temperature observed in some depressed individuals may also be responsible for the disturbance in TSH secretion.

Treatment of Depression by Sleep Deprivation

In our earlier trial of partial sleep deprivation, we found that the therapeutic effects of a single night of sleep deprivation could be extended if it was followed by second night treatment and the therapeutic effects of two nights of treatment were sustained through a night of recovery sleep. Following several additional nights of recovery sleep patients usually relapsed to baseline. These observations led us to hypothesize that a longer course of PSD might have a cumulative effect and result in complete clinical remission. As part of our current investigation, we have treated 7 depressed inpatients with repeated partial sleep deprivation. Of these seven, 2 improved, one with a complete remission of her depressive symptoms. In the other five there was no change in their clinical condition. These preliminary results have failed to fulfill our expectations but this may in part due to the relatively refractory group of patients who are referred to our program (three of the seven patients studied were ultimately treated with ECT after failing treatment with antidepressants). An adequate evaluation of repeated partial sleep deprivation therapy may require a more treatment responsive population and we considering alternative experimental designs at the time.

Theoretical Considerations

There is accumulating evidence from our circadian studies that temperature and HPT regulation is disturbed in depression. These changes may be part of a larger disturbance of energy and metabolism which would account for the weight and activity changes seen in these patients. The seasonal incidence of suicide and mania may reflect a diminished capacity of the organism to adapt normally to thermal stresses.

Patients undergoing sleep deprivation therapy are subjected to a thermal stress at a particular time in their circadian cycle. The beneficial effects of this treatment may be mediated by heat loss and the compensatory activation in TSH secretion this evokes. In future studies, we plan to directly assess the basal metabolic rates of our depressed patients, its relationship to basal temperature and its change with sleep deprivation. The relationship between thermoregulation and the antidepressant effects of sleep deprivation will also be systematically explored in future studies.

Section on Sleep Studies

Wallace B. Mendelson, M.D.

The sleep laboratory has continued to pursue clinical and basic science issues related to affective disorder, insomnia and pharmacology of sleep. Because of interest in changes in sleep such as short REM latencies and possible alterations in delta EEG power, we examined sleep and EEG power spectra in a primarily bipolar population. Although some of the stigmata of depression were present, there was no evidence of short REM latency, and no difference between patients and controls in EEG delta power. The former finding, in agreement with four recent studies, continues to suggest fundamental differences between unipolar and bipolar depression. The latter calls into question theories of depression built on the assumption of alterations in EEG power.

Our studies of insomniacs this past year have indicated that "poor" sleep is not necessarily "light" sleep; i.e., insomniacs do not awaken more easily than insomniacs return to sleep and stay asleep after experimental interruptions as easily as controls. They did, however, underestimate the amount of sleep they obtained by 50%, suggesting once again that they suffer from a misperception of their state of consciousness.

The laboratory has continued investigations suggesting that the hypnotic effect of benzodiazepines is mediated by alterations in calcium flux. We have examined the possible interaction of agents which affect calicum flux with anxiolytic and anticonvulsant effects of diazepam. It was determined that the properties of nifedipine which we previously described are specific to sleep induction, suggesting that sleep alterations by benzodiazepines may have a very different mechanism than their hypnotic properties may involve other ionic mechanisms.

We have expanded our previous work showing that chloride channel blockers may greatly reduce toxicity of barbiturates. This year the focus has been on respiration of rats and have established curves showing decreased respiration of rats and have established curves showing decreased respiration after giving pentobarbital and flurazepam. The next step will be to examine agents which may alter the effects of barbiturates and benzodiazepines on respiration. In principle, such work might lead to compounds which would greatly reduce mortality from overdoses of sleeping pills.

Biology of Melatonin and Neuroendocrinimmunomodulation

Lawrence Tamarkin, Ph.D.

During the past year we have made a major shift in the direction of our laboratory investigations. For the last 14 years I have been investigating the biology of the pineal gland and its hormone melatonin. These investigations have reached a point where I feel I need to step back and critically evaluate our progress. During this last year we have made some interesting clinical observations on the daily profile of melatonin (project #Z01 MH 02290-02 CP) and perhaps have seen a demonstrable effect of melatonin on tumor cell growth in athymic, "nude" mice (project #Z01 MH 02292-02 CP). However, the overall productivity of these studies has been slow and frustrating. I feel we have not been able to resolve some critical questions and more importantly, I have felt that more fruitful avenues of research were passing us by.

I have been fascinated with the interactions between the CNS and the immune system. The classic behavioral studies on stress and immune function, as it applies to life expectancy or tumorigenesis is of particular interest to me. Specifically, we have looked at these questions from an endocrine perspective. That is, the intervening signals from the brain to the immune system most probably are endocrine hormones. Conversely, the immune system may be communicating to the brain via proteins that are secreted by immune cells, specifically the lymphokines and cytokines.

We have initiated our investigations to include clinical and basic *in vivo* and *in vitro* studies. To investigate a behaviorial/environmental impact on the immune system we have been evaluating the responsiveness of human and rodent lymphocytes before or after "bright" light exposure. Also, we have evaluated peripheral blood lymphocyte activity during social group inteactions in the rhesus monkey.

We have coupled these behavioral studies with specific neuroendocrine challenges to determine the effect on rat splenic lymphocytes. These studies have given us insight into specific endocrine modulation of lymphocytes and also allow us to directly study mechanisms of action of hormones on rat lymphocytes. We have also proposed the hypothesis that lymphokines, specifically interleukin 1 and 2, may act on non-immune cells. Essentially, these proteins may be similar to other circulating hormones. We have observed that IL-1 and IL-2 can directly affect the growth of cultured human breast cancer cells. For IL-2 the effect is biphasic with inhibition of growth at high doses and stimulation of growth at low doses. The implication of this observation is that factors that may lower immune function, and consequently lowering IL-2 production, may actually facilitate tumor cell growth.

Lymphokines may have direct effects on non-immune, non-cancer tissue as well. We have preliminary data which indicate that IL-2 can cause the release of adrenocorticotropin from cultured anterior pituitary glands. This possibily is a new homeostatic feedback loop involving positive feedback of IL-2 on ACTH release, which in turn causes a release of adrenal steriod which would suppress lymphocyte activity.

The study of the biology of IL-2 independent of the immune system is a novel question, and we have received 25 mg of IL-2 (the equivalent value equal to approximately $1 million dollars) as a gift from the Cetus Corp. to pursue this question. With this amount of material we now have the capability of investigating a broader scope of this question. I believe we now have developed a number of lines of investigation to approach the question of the bidirectionality of the endocrine and the immune systems and its regulaton by the brain.

Antidepressant Pharmacology of the Rodent Circadian System

Wallace C. Duncan, M.S.

Severe sleep disturbance is a major symptom of depressive disorders; this complaint may be quantitatively described as hypersomnia or hyposomnia, or qualitatively described as the inability to fall asleep in the evening, remain asleep in the morning, or the urge to nap during the daytime. These symptoms are characteristics of a poorly regulated circadian system which may manifest as disturbances of the activity-rest cycle or of other physiological processes such as thermoregulation and hormone release. The breadth of these dysfunctions through a variety of physiological systems suggests that a basic flaw exists at a primary nucleus of the human circadian system. The primary candidate for this lesion is the suprachiasmatic nucleus of the hypothalamus. This central circadian clock is responsible for controlling the frequency and timing (phase) of mammalian biological rhythms. Defects in these parameters may underlie many of the physiological and psychological disturbances which accompany depression; treatments which affect these two parameters may participate in the therapeutic antidepressant response.

Two converging lines of evidence suggest that manipulation of the human circadian system is related to a clinical antidepressant response. First, pharmacological studies have indicated that the psychoactive compound, lithium, which may exhibit antidepressant effects after several weeks, alters the frequency of the central clock in a variety of invertebrate and vertebrate species, including humans. Second, non-pharmacological phase manipulations of the activity-rest cycle such as sleep deprivation or phase advance therapy have been found to produce an antidepressant response within a few days. Thus, both pharmacological and non-pharmacological interventions that involve manipulation of the circadian system have been observed to elicit clinical remission of depressive symptoms; a basic distinction between these two circadian interventions is the time course required for clinical remission.

If the phase change is critical for the antidepressant response, one would predict that manipulations which clearly alter the phase relationship between the central clock and the activity-rest cycle, i.e. phase advance and sleep deprivation therapies, would produce a more rapid antidepressant response than treatments which fail to alter this phase relationship. We hypothesized a) that the antidepressant mechanism of chemical antidepressants was related to their effects on the circadian clock, specifically to changes in the frequency of the central clock, and b) that since chemical antidepressants have delayed therapeutic effects, the phase relationship between the clock and the activity-rest cycle would be conserved to a greater degree during pharmacological treatments than during non-pharmacological treatments.

The distinction between a) the frequency of the central circadian clock and b) the phase relationship between the central circadian clock and the activity-rest cycle, has been a neglected area in psychiatric chronopharmacological research. Such discrimination has been difficult until now because of the time and effort required to measure these parameters of the mammalian circadian system. However, we have recently designed and constructed a computer-based rodent circadian facility (RCF) which permits the rapid assay of a) the central circadian clock, and b) the phase relationship between the clock and the activity-rest cycle.

To test our hypotheses we have designed several experiments and have been evaluating the effects of the chemical antidepressant, clorgyline, an irreversible monoamine oxidase inhibitor (MAOI), on frequency and phase parameters of the circadian system in the Syrian hamster. In addition, we have recently obtained preliminary data regarding this compound's effects on feeding behavior and body mass.

We have found chronic clorgyline administration to produce three dramatic effects on the rodent circadian system. First, clorgyline significantly decreases the frequency of the circadian clock, as does lithium, and supports our first hypothesis that the central circadian clock is a target for chemical antidepressants. Second, clorgyline produces a dramatic increase in the activity-rest ratio, thus depriving the hamster of part of its rest phase. Third, consistent with our second hypothesis, we have found that this compound conservatively alters the phase relationship between the central circadian clock and the onset of the activity-rest cycle.

Thus there appears to be a fundamental difference between the organization of the circadian system during chronic clorgyline treatment and during sleep deprivation or phase advance therapy. Specifically, during clorgyline treatment, the activity-rest cycle exhibits a small phase-advance relative to the circadian clock, whereas during sleep deprivation or phase-advance therapy this phase relationship is, by definition, dramatically altered. This difference may explain the different time courses of remission observed for these two modes of treatment. These data offer compelling evidence that MAOI antidepressant, clorgyline, affects the mammalian circadian clock. We are currently investigating a) the specific MAOI properties of this compound that are responsible for these effects and b) the effects of other psychoactive compounds on the hamster circadian system.

In addition, we have preliminary data that clorgyline decreases food intake and body mass in the Syrian hamster. Together with our finding that clorgyline deprives the hamster of part of its rest period, these observations may ultimately prove to be relevant to clorgyline's utility in the treatment of hypersomnic/hyperphagic type depressions.

In summary, we have demonstrated the MAOI, clorgyline, alters properties of the mammalian circadian clock. Our observations are consistent with the hypothesis that a central dysfunction in this clock may underlie some of the major symptoms of depression and that further, the mechanism and clinical course of the pharmacological antidepressant response may be related to specific properties of the circadian system. The computer-based RCF will greatly enhance our ability to further unravel the circadian basis of the pharmacological antidepressant response.

ANNUAL REPORT OF THE LABORATORY OF CLINICAL SCIENCE
NATIONAL INSTITUTE OF MENTAL HEALTH
October 1, 1985 through September 30, 1986

Dennis L. Murphy, M.D., Chief

The Laboratory of Clinical Science is in its second year of evolution following its inception via the merger of three laboratories last year. It is still a coalition of several fairly senior, quite autonomously functioning sections and newer, developing groups. Sufficient common interests and a good general spirit of cooperation, mutual support and generous, concerned critique continue through formal seminars and informal contacts, providing common benefits to all. Some centralized administrative and procurement services also generally facilitate management functions for the sections and mediate interactions with the NIMH-IR administrative and personnel offices.

As indicated in the individual section summaries presented below, a continued high level of productivity has been maintained in both the groups with a primary clinical focus headed by Drs. Potter, Jimerson and Murphy, as well as in more basic sections and units directed by Drs. Markey, Jacobowitz, Insel and Saavedra. A significant addition this past year was the strengthening of the HPLC analytic group in the Section on Clinical Pharmacology resulting from Dr. Ivan Mefford's most welcome recruitment into the section. The greatest changes in the offing involve the Section on Comparative Studies of Brain and Behavior, with the upcoming strengthening of NIMH-NICHD program at Poolesville by the transfer of Dr. Steven Wise's highly regarded and established program from Building 31 to the Poolesville NIHAC and the addition of more neurochemical strength through the move to Poolesville of Dr. Jan Johannessen.

Section on Analytical Biochemistry
Sanford P. Markey, Ph.D., Chief

This Section conducts research on the development and application of analytical procedures and instrumentation to problems in neuropharmacology. During the past year, research effort was divided mainly between new instrumentation (a Fourier transform mass spectrometer) and studies on the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine).

During earlier studies, Drs. Johannessen, Chiueh and Markey defined the toxicity of MPTP in primates, dogs and rodents. MPTP produces a permanent parkinsonian syndrome in primates which closely resembles both the syndrome found in MPTP-exposed humans and idiopathic Parkinson's disease. Biochemically, the lesions produced by MPTP are somewhat more specific and circumscribed than those found in Parkinson's disease, principally being a loss of dopaminergic neurons in the A-9 brain region. In the sub-human primate, the consequence of this cell loss is a profound movement disorder, but in the dog, compensatory mechanisms permit animals to fully regain their ability to move normally. In the rodent, neither permanent cell loss nor locomotor deficits result from MPTP, apparently due to neuronal regrowth and compensatory mechanisms. Present research has been directed at understanding the differences in neurotoxicity and metabolism of MPTP in various species, finding MPTP-like compounds which would permit sub-cellular localization of MPTP and MPTP-metabolites, and devising methods to detect environmental agents related to MPTP which might be causative factors in idiopathic Parkinson's disease.

For these studies, amino-substituted analogues of MPTP were prepared and tested for neurotoxicity in mice and dogs. The 4'-amino-MPTP analogue has MPTP-like effects in that it decreased dopamine content in mouse striatum and dog caudate. Future studies will define this effect further, and the suitability of this analogue for sub-cellular localization will be determined. The amino analogues were also used to synthesize antigens both for the production of antibodies by rabbits and for the development of an enzyme-linked immunosorbent assay (ELISA). The ELISA technique will be used to screen tissue extracts from brains of patients with idiopathic Parkinson's disease and Guamanian parkinsonism dementia for compounds structurally related to MPTP.

$^{14}C_6$-labelled MPTP has been synthesized by Dr. Yang and is being used to study MPTP metabolism in various animal species. Two brain metabolites have been characterized, but more remain to be identified by chromatographic and mass spectrometric methods. Substances which inhibit or enhance the toxicity of MPTP will be studied with ^{14}C-MPTP to determine if their effects are due to alterations in metabolism. A quantitative technique for measurement of MPP+ in tissue samples by high performance liquid chromatography has been developed, and will be expanded for use with MPTP and other metabolites as they are identified.

One batch of ^{13}C-labelled norepinephrine has been prepared by Dr. Weisz and purified by a novel counter current chromatographic method. This synthesis will be repeated to prepare sufficient material for human clinical studies.

Collaborative studies on the role of melatonin in human physiology are continuing. Several clinical projects utilize melatonin metabolite levels as an integrative measure of beta-adrenergic activity. The mass spectrometric assay of 6-hydroxymelatonin remains the most useful integrative measure of pineal function for animal or human studies.

The mass spectrometric instrumentation projects have been markedly altered in the past year. The high performance Fourier transform spectrometer received last year has been thoroughly tested and found to have several major design flaws. As the first of its kind, some difficulties were anticipated, but the nature and extent of the problems has necessitated redesign of several components by the manufacturer. Consequently, useful analytical applications have not been possible. Resolution of these problems is anticipated during this year. The second major collaborative project, the surface imaging instrument being built at NIH with support from NHLBI and NIMH and personnel from BEIB has been terminated due to the departure of Dr. L. Kelner. The instrument equipment will be transferred to Oak Ridge National Labortory where a new collaboration will begin with Dr. Peter Todd. In another collaboration, studies continue with the microwave powered reaction-interface with Dr. F. Abramson at George Washington University. Small quantities of sulfur have been quantified, and a recently completed interface will be applied to ^{14}C detection of MPTP metabolites in the future.

Section on Biomedical Psychiatry
David C. Jimerson, M.D., Chief

Clinical studies during the past year have continued to focus on the psychobiology of the eating disorder syndromes of anorexia nervosa and bulimic disorder. This research has included neurotransmitter, neuropeptide, neuroendocrine, metabolic, pharmacologic and related behavioral studies, with a goal of understanding neurobiologic factors contributing to the etiology and variable responsiveness to treatment of these disorders. The studies have involved present Section staff (Drs. Brewerton, Brandt and Lesem, Ms. Kassett, Dr. Obarzanek and Dr. Jimerson), previous members of the Section (Drs. Kaye, Gwirtsman and George) and a number of other collaborators in LCS and other branches of the NIMH Intramural Program.

Design for the clinical studies has included longitudinal assessment of individual patients with eating disorder symptoms, as well as cross-sectional comparison with healthy volunteers and with other psychiatric patients (especially patients with affective and anxiety disorders being studied by other NIMH investigators). Because of the likely influence of altered dietary patterns and various degrees of weight loss on neurobiologic studies, anorexic patients have been studied at low weight during a period of nutritional and metabolic stabilization, and then during progressive phases of weight restoration during treatment. Similarly, non-anorexic patients with bulimic disorder have been studied immediately after admission to assess the neurobiologic milieu accompanying chronic bingeing and vomiting, as well as after a binge-free period of nutritional and metabolic stabilization during hospital treatment. Preliminary pharmacologic treatment studies in bulimic patients have also been conducted in the new outpatient eating disorder clinic. Structured interviews with bulimic patients and their first degree relatives have been initiated for a family study (conducted in collaboration with Dr. E. Gershon) to identify relationships to other psychiatric disorders.

Neurotransmitter studies over the past year have provided new evidence for altered serotonin function in patients with bulimia, consistent with the hypothesis that functionally decreased central serotonin function could contribute to symptoms of impaired post-prandial satiety, depressed mood and behavioral impulsivity. Preliminary results in bulimic patients at normal weight demonstrated blunted prolactin responses to the serotonin receptor agonist m-chlorophenylpiperazine (m-CPP) (studied in collaboration with Drs. D.L. Murphy and E.A. Mueller), and a similar trend toward blunted prolactin responses to the serotonin precursor L-tryptophan. Initial results have also demonstrated blunted prolactin responses to L-tryptophan in the first several low weight anorexic patients studied. Bulimic subjects, particularly those with a personal/family history of migraine headaches, showed increased sensitivity to severe migraine-like headaches following m-CPP administration, providing new evidence for a role of serotonin in migraine, and suggesting that vascular serotonin receptors may be altered in some bulimic patients. Evidence for altered central serotonin activity in bulimic patients would be consistent with the hypothesis that antidepressant drugs may decrease symptoms in some bulimic patients through serotonergic effects. In further support of this hypothesis, in the first several patients studied in a double-blind outpatient trial, we have observed beneficial effects with the serotonergic agonist fenfluramine.

Followup studies on norepinephrine function provided data on state-dependent alterations in sympathetic nervous system activity in eating disorder patients.

In the immediate post-admission phase, bulimic patients participating in a naturalistic physiologic study of bulimic episodes demonstrated relatively normal basal levels of plasma norephinephrine, with large increases following binge eating. The true resting activity of the sympathetic system appears to be low in these patients, however, since hospitalization with abstinence from bingeing and vomiting for at least three weeks resulted in a decline of plasma and CSF norepinephrine levels to values significantly lower than control levels. Further studies are in progress to assess whether reduced sympathetic activity in these patients is related to low caloric intake and slightly subnormal body weights. In collaboration with Drs. S. Paul and D. Pickar, we are also evaluating sympathetic nervous system responses (as well as neuroendocrine and behavioral responses) to metabolic/hypoglycemic challenge with 2-deoxyglucose in bulimic patients. In low weight anorexic patients, previous studies have reported reduced plasma norepinephrine levels. In data from recently studied patients, we have found that plasma norepinephrine levels are in a normal range in acutely cachectic patients immediately following admission, with a subsequent decrease during nutritional and metabolic stabilization. These results suggest that decreased sympathetic activity expected to accompany weight loss may be obscured in cachectic anorexic patients by such other illness-related factors as reduced intravascular volume or illness-related stress.

New studies to clarify the role of endogenous opiates in anorexia and bulimia have been initiated over the past year. We have collected lumbar cerebrospinal fluid samples in these patient groups to evaluate clinical correlates of our previous finding of reduced levels of beta-endorphin and other pro-opiomelanocortin related peptides in low weight anorexic patients, and to extend these studies to other endogenous opiate peptides. We have begun behavioral and neuroendocrine studies with high dose infusions of naloxone in eating disorder patients (in collaboration with Dr. D. Pickar). Preliminary results suggest a paradoxical effect of naloxone on consumption of a test meal in anorexia nervosa, with several low weight patients showing increased calorie consumption following drug pretreatment, in contrast to a decrease in food intake produced by naloxone in obese subjects and other patient groups. These studies are of particular importance in evaluating the possible role of opiate antagonists in the treatment of anorexia and bulimia.

Collaborative studies with Dr. P. Gold on regulation of the hypothalamic-pituitary-adrenal (HPA) axis have continued over the past year. Dr. Gold published results showing normally restrained pituitary responsiveness to infusions of corticotropin releasing hormone (CRH) in hypercortisolemic, low weight anorexic patients, indicating that increased activity of the axis in these patients originates at the level of the hypothalamus or more rostral brain sites. In a related study, measurement of CRF in lumbar CSF samples has demonstrated increased concentrations of the peptide in low weight anorexic patients, consistent with results of the CRH study, and with other previous reports suggesting central dysregulation of the HPA axis in this patient group. This finding is of particular interest because of the anorexic effects of CRH observed in laboratory animals. We have now initiated a new series of collaborative studies with Dr. Gold evaluating neuroendocrine responses to a glucocorticoid antagonist in eating disorder patients.

In studies on energy metabolism in eating disorder patients over the past year, we have demonstrated that bulimic patients at normal weight are able to maintain a constant body weight with a smaller caloric intake than healthy controls. This finding is an extension of our previous observation that anorexic patients with bulimic symptoms require fewer calories to maintain their weight than do non-bulimic anorexics. These findings are of importance because they provide one physiological explanation for the complaint of many bulimic patients that they find themselves particularly prone to weight gain on a typical diet. We are presently comparing metabolic rate, as assessed by indirect calorimetry, with clinical variables (e.g., duration and severity of symptoms, family history of eating disorders, etc.) to assess possible causes contributing to altered energy metabolism in these patients. We have also observed during longitudinal assessment of energy metabolism in anorexic patients that metabolic rates increase significantly during refeeding, accounting in part for the large caloric requirements for weight gain in many of these patients.

Laboratory work this year involved several collaborations directed toward further assessment of the interaction between the sympathetic nervous system and the HPA axis in psychiatric subjects. In one study with Drs. Wolkowitz and Pickar, we observed that dexamethasone produced different effects on plasma MHPG levels in patients with severe depression and in controls, extending previous evidence for altered corticosteroid-catecholamine relationships in depression. Other laboratory work was focused on assays to support the clinical studies described above--e.g., measurement of plasma prolactin by radioimmunoassay and measurement of plasma tryptophan levels by high pressure liquid chromatography. In a series of studies on the behavioral pharmacology of feeding begun during the past year, Dr. J. Stucky has started experiments to evaluate behaviorally and pharmacologically induced alterations in central opiate and monoamine activity as a basis for animal models of anorexia and bulimia.

Section on Clinical Neuropharmacology
Dennis L. Murphy, M.D., Chief

This Section explores the biochemical and behavioral pharmacology of novel as well as some standard psychoactive agents in attempts to both understand how these drugs work and, more importantly, to use these drugs to investigate normal and abnormal brain neurochemistry. While maintaining a base of studies aimed towards the comparative examination of the mechanisms by which antidepressant drugs act, we have extended our clinical studies into non-primary affective disorder clinical populations, particularly obsessive-compulsive disorder and Alzheimer's disease to explore affective or affective-related components of these disorders.

These two latter disorders, at least during some phases, exhibit psychobiologic abnormalities similar to those originally thought to be specific markers of the affective disorder. Obsessive-compulsive disorder responds to the tricyclic antidepressant drug clomipramine, even in the absence of clinically definable depression, and, at least in some patients, antidepressant drugs may benefit Alzheimer's patients. To clarify the relationship of affective symptomatology in Alzheimer's disease to typical affective disorder, the section has embarked on several comparative studies involving Alzheimer's patients, elderly depressed patients (a population somewhat neglected in other NIMH-IR affective disorder studies) and age-matched normal controls.

The Unit on Geriatric Neuropharmacology established last year under the leadership of Dr. Trey Sunderland has developed new assessment measures for these subject populations. A series of studies using cholinergic agents as probes of the functional status of this neurotransmitter system in Alzheimer, depressed and normal populations have reached various stages of completion. The first direct comparison between Alzheimer patients and age-matched controls revealed an enhanced sensitivity to the cholinergic antagonist, scopolamine, in Alzheimer patients, verifying the significance of the previously found similar difference between Alzheimer patients and younger normal controls. Interestingly, physiological responses to scopolamine were no different, Interestingly, while behavioral and cognitive supersensitivity was quite striking in the Alzheimer patients, physiological responses to scopolamine were no different. Extension of this study to depressed patients will be completed shortly, with major assistance from Dr. Paul Newhouse.

Studies examining the opiate antagonist, naloxone, also found major abnormalities in responses of Alzheimer patients compared to controls in a study led by Dr. Pierre Tariot. Dr. Tariot is also completing data analysis and final reports on investigations of the cognitive, behavioral and physiological affects of the cholinergic agonist arecholine, and of the monoamine oxidase (MAO)-inhibiting agent with selective MAO-B effects, deprenyl, given chronically to Alzheimer patients. Dr. Newhouse has also made substantial progress in a clinical study of a wide range of doses of another cholinergic agonist, nicotine, in normal volunteers and Alzheimer patients. This work in geriatric psychopharmacology has been the subject of several major symposia at national meetings as well as a joint NIMH intramural/extramural program symposium in June, 1986.

Akin to manifold approaches of the strategy to use pharmacologic probes to assess the status of the cholinergic and opiate brain neurotransmitter/neuromodulator systems in populations where these systems may be involved in symptomatology, we have been engaged in a four-year program to develop, refine and critically evaluate a similar strategy to assess the functional status of the brain serotonergic neurotransmitter system in neuropsychiatric disorders. We have established an extensive program in basic neurochemistry, rodent behavioral pharmacology (under the direction of Dr. Charanjit Aulakh), non-human primate studies, and direct clinical studies (coordinated by Dr. E.A. Mueller) primarily focused on the first serotonin receptor agonist available for study in humans, m-chlorophenylpiperazine (m-CPP). Neuroendocrine (prolactin, cortisol, ACTH), temperature, cardiovascular and behavioral endpoints including mood, locomotor activity and feeding behavior have all been examined. Changes in response to m-CPP are being evaluated in relationship to different pharmacologic and behavioral manipulations.

In clinical studies, m-CPP has begun to be used as a probe to compare brain serotonergic function in five different neuropsychiatric syndromes, as well as to assess the consequences of psychoactive drug treatment on serotonin function. Serotonergic contributions to antidepressant and other psychoactive drug effects have long remained enigmatic, in part related to the lack of selective agonists and antagonists for the multiple serotonin receptors in brain, and this current set of studies seem to be a promising approach to clarify serotonergic function as has been recently accomplished (at least in part) for noradrenergic and dopaminergic adaptational events during antidepressant treatment.

A pertinent clinical example is developing from this year's studies of m-CPP in obsessive-compulsive patients. m-CPP induced acute, short-term enhancement of obsessive-compulsive symptoms which did not seem to simply represent an increase in non-specific anxiety, but rather symptoms typical of the disorder itself. These changes were in quite marked contrast to relatively minor or non-existent behavioral or mood changes in 15 normal volunteers. These results may be related to the unusual responsiveness of these patients to clomipramine (and, in recent studies, to other agents with selective actions on serotonin uptake such as fluvoxamine and fluoxetine), while other tricyclic antidepressants such as desimpramine seem less effective. These and other studies reviewed by Drs. Zohar and Insel led to their receipt of the Society of Biological Psychiatry's Bennett Award in June, 1986.

Section on Clinical Pharmacology
William Z. Potter, M.D., Ph.D., Chief

The Section operates as three independent but interacting units: a Clinical Research Unit (Dr. Matthew Rudorfer), a Unit on Clinical Neurochemistry (Dr. Ivan Mefford), and a Unit on Preclinical Neuropharmacology (Dr. Juan Saavedra). The focus of the Section is on the mechanisms of action of drugs affecting the central nervous system. We employ a wide variety of techniques but specialize in measures of neurotransmitters and their metabolites in accessible tissues from humans and in specific brain regions from animals.

The Clinical Research Unit continues to investigate an organizing hypothesis that patients with affective illness have a dysregulation of neurotransmitter systems which is "re-regulated" by antidepressant treatments. Noradrenergic dysregulation continues to be found in depressed patients most consistently as elevated plasma norepineprhine (NE) to the physiologic stress of standing. Possible, but less consistent dysregulation of the serotonin and/or dopamine system is newly found in an elevated prolactin response to intravenous chlorimipramine. We have now shown electroconvulsive therapy (ECT) consistently reduces plasma NE, in the predicted direction of "re-regulation." Unexpectedly, ECT does not reduce MHPG in the cerebrospinal fluid (CSF) but does increase HVA, the major dopamine (DA) metabolite and/or 5HIAA, the major serotonin (5HT) metabolite. To our knowledge, no antidepressant drug has these effects; in fact, most drugs which we have studied consistently reduce 5HIAA in CSF. Moreover, since ECT is the one treatment which works in psychotic depression, we hypothesize that the increased HVA reflects an action of ECT on the DA system which mediates the antipsychotic effects.

The findings of complex treatment effects on CSF amine monoamine metabolites led us to work on inter-relationships between measures. We have followed up on our finding that the data in man and animals supports a positive influence of 5HT on DA turnover as measured by 5HIAA and HVA in CSF of humans as well as a fluctuating mutual interdependence of NE and 5HT in brain regions of rats (see below). We find that between individuals either in normal or overall patient groups, there is a high positive correlation of 5HIAA and HVA and a modestly high ($r = 0.5$) correlation between MHPG and 5HIAA. Between patients who respond to treatments these relationships are particularly evident; in 20 total nonresponders these significant correlations are absent. We suggest that this finding may document a degree of dysregulation of interactive monoamine systems which underlies poor therapeutic response and may therefore have both practical and theoretical implications.

The Unit on Clinical Neurochemistry develops new high performance liquid chromatographic (HPLC) methods and investigates neurotransmitter effects of treatments in animals as well as running a "Central Neurochemistry Service" providing assays for IRP clinical research programs. Method development has yielded progress in these areas:

1. Surfactants and selectivity. The use of nonionic surfactants in the mobile phase allows one to introduce hydrophilic binding sites onto a reverse phase column while decreasing the hydrophobic interactions. This allows separation of relatively non-polar compounds without the use of organic modifiers in the mobile phase. When this approach is combined with ionic surfactants, novel separations of charged species such as catecholamines are obtained in which secondary hydrogen bonding dictates the order of elution.

   As a result of the above observations, the ionic surfactant containing a polar atom adjacent to a sulfonic acid functional group was used as an ion pairing reagent. This compound, N-methyl oleoyltaurate had properties quite similar to mixed ionic/nonionic surfactants and allowed separation and detection of all three catecholamines in blood plasma in ten minutes.

2. Only a small fraction of chemical compounds can be oxidized or reduced within the limits imposed by the oxidation and/or reduction of water, the normal solvent in reverse phase HPLC. Although results are still fairly preliminary, the use of an electroactive ion-pairing reagent, diisopropylnaphthyline sulfonic acid or anthraquinone sulfonic acid, allows one to detect any cation through "vacancy" chromatography. This approach has been applied to several inorganic cations, Li^+, Na^+, K^+, NH_4^+, Ba^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Ag^+, Pb^{2+} with good success.

   The use of vacancy electrochemical detection will allow development of sensitive assays for non-electroactive compounds such as GABA, taurine, acetylcholine, PEA and others. Detection limits should be in the picogram range.

3. Equations were derived and tested to demonstrate their validity. These equations accurately predict peak current and show that microbore HPLC coupled with amperometric detection can give detection limits in the 20-50 fg (100-300 attomole) range.

Studies on treatment induced biochemical alterations have focused on ECT and insulin coma which result in depletion of hypothalamic epinephrine. Epinephrine is more drastically depleted than is norepinephrine. Chronic clorgyline leads to a more marked increase in hypothalamic epinephrine than norepinephrine.

The "Central Neurochemistry Service" has functioned with increasing efficiency processing over 6,000 samples of CSF, plasma and urine for neurotransmitters and their metabolites. We are currently in the process of entering all CSF values on a central computer file to be available for all clinical groups so as to follow trends over time, control values, variations across groups, etc. The standardization of procedures is critical to the validity of this effort and is being achieved through an interactive process of feedback of values to specific investigators influencing submission of subsequent samples.

The Unit on Preclinical Neuropharmacology has moved forward in the study of regionally specific monoamine and peptidergic receptors possibly involved in physiologically relevant neurotransmission. Several studies derive from an ongoing interest in central control of blood pressure since there is a readily available animal model and since there is such an overlap between substances involved in this regulation and those involved in the action of psychotherapeutic drugs. Ten findings and/or directions for future research can be briefly summarized.

1. Quantitative autoradiographic methods have been further developed and utilized to characterize receptors for neuropeptides (angiotensin and atrial natriuretic factor), biogenic amines (dopamine D1) and peptidases (angiotensin converting enzyme, kininase II) in individual brain nuclei from single rats. These methods are an order of magnitude more sensitive than classical binding techniques and have the additional advantage of the preservation of anatomical structures allowing for the study of specific nuclei and brain tracts.

2. These techniques can be applied to the localization and characterization of multiple receptors in individual nuclei from single human brain autopsy specimens.

3. Both kininase and D1 binding have been localized to the striato-nigral pathway, suggesting interactions between neuropeptide and dopmaine systems.

4. We identified a brain circuit of angiotensin receptors which represents the connection between the peripheral and the brain angiotensin systems. This circuit is important for the central regulation of blood pressure and fluid metabolism.

5. Angiotensin interacts with catecholamine neurons in brain and with the peripheral sympathoadrenal system at many levels, indicating the possibility of clinically important interactions between the renin-angiotensin and the sympathetic systems.

6. Receptors for the atrial natriuretic factor, a circulating hormone produced by the heart, are low in the brain of hypertensive animals. This finding indicates a central role for this peptide. The use of atrial natriuretic factor analogs may be of benefit for the treatment of essential hypertension in man.

7. Conversely, increased angiotensin receptors occur in the brain of hypertensive animals, indicating an antagonism between angiotensin and atrial natriuretic peptide systems in brain.

8. Some of the ligands used could be considered as new potential candidates for brain imaging in many, D1 antagonists and kininase inhibitors to image the striato-nigral complex and related structures, the atrial natriuretic factor and kininase inhibitors to image the choroid plexus, and angiotensin to image circumventricular organs and chromaffin cells in the adrenal medulla and parachromaffin tissues.

9. A D1 antagonist binds to melanin, primary melanomas and their metastases in both animals and human tissues. This or related compounds have potential use in the diagnosis, localization and treatment of human melanomas. Autoradiographic techniques could be utilized in human pathology for the localization, quantification and classification of tumors in biopsy specimens.

10. Detailed studies on biogenic amine metabolism in multiple nuclei of the rat brain revealed correlations between the serotonin and the catecholamine metabolism of possible importance in the regulation of circadian rhythms. These findings could be used to further understand changes in amine metabolites in human CSF, and substantiate clinical findings of a close correlation between the metabolism of catecholamines and serotonin in man.

Section on Comparative Studies of Brain and Behavior
Dennis L. Murphy, M.D., Acting Chief

This relatively new section, based at the NIH Animal Center in Poolesville, Maryland was formed last year from a convergence of three interests: Paul Maclean's original Laboratory of Brain Evolution and Behavior, Steve Suomi's new NICHD Laboratory of Comparative Ethology and Tom Insel's previous work in the LCS studying animal models of psychopathology. The resulting Section reflects all three sources including MacLean's commitment to comparative neuroanatomy, Suomi's focus on non-human primate behavior and development, and Insel's interest in the neural substrates of anxiety. The Section has evolved to focus primarily on developmental psychobiology with studies of mother-infant attachment behavior in rodents and non-human primates. The following questions are being pursued:

1. What are the neural pathways involved in the behavioral response to mother-infant or peer grop separation?

2. What are the neural substrates for parental behavior?

3. What are the long-term consequences of early experiences of separation or stress?

For studies of the acute response to separation, isolation calls were recorded from infant rats and squirrel monkeys. As the rat pup isolation calls are ultrasonic, a novel computer-based system was developed for analyzing and displaying their number and frequency range. This technique revealed an increased calling rate in two-day-old pups from an inbred strain of rats selected for fearfulness (Maudsley Reactive strain). In pharmacologic studies in both rodents and squirrel monkeys, agents affecting brain benzodiazepine, opiate and adrenergic systems were potent modulators of isolation calls. Specifically, agents which are clinically anxiolytic were found to reduce the calls. Lesion studies continued to implicate a narrow band of limbic cortex in the mediation of the squirrel monkey isolation calls. Using a retrograde tracing technique, this area was shown to receive afferents from select thalamic nuclei. The robust and reproducible nature of isolation call in rodents and non-human primates recommends its use for further studies of mother-infant or peer group separation. Studies currently underway will explore local changes in the binding of benzodiazepine and opiate ligands during separation with the use of an *in vivo* autoradiographic technique.

Studies of attachment behavior from the parental side demonstrated that oxytocin, a hypothalamic peptide long known to induce uterine contraction and milk let-down, also has a role within the central nervous system for the mediation of maternal behavior. When oxytocin was administered into the lateral cerebral ventricle of nulliparous, virgin, ovariectomized female rats, full maternal behavior was observed. This effect was shown to interact with olfactory cues, with oxytocin appearing most potent in anosmic females. A physiologic role for oxytocin in maternal behavior was investigated by identifying and quantifying receptors for this peptide in various brain regions using in vitro receptor autoradiography. Oxytocin receptors, although extremely limited in brain, increased in discrete regions following parturition. These discrete changes could be reproduced by ovariectomy and estrogen administration, a treatment that mimics the endocrine changes prior to parturition and has been previously shown to facilitate maternal retrieval in nulliparous females. We hope to extend these studies with oxytocin to a non-human primate, the pygmy marmoset. During this past year, Dr. Wamboldt has established that males are responsible for the majority of parental care in this species, providing an opportunity for the study of peptide effects on parenting independent of the influence of estrogen.

Another brain peptide, corticotropin releasing factor (CRF), was studied for its effects early in development. Brain receptors for CRF were identified by 17 days of gestation in the rat fetus, with a peak in receptor number at eight days post-partum. Repeated treatment with CRF during the first week of life did not induce significant acute or chronic behavioral changes, but from preliminary evidence it appears that neonatal treatment with CRF may increase activation of the pituitary-adrenal axis during stress in adulthood. Similar long-term consequences of neonatal peptide administration were described previously by Dr. Handelmann for opiates, substance P and vasopressin.

In yet another paradigm to study the long-term consequences of early experiences, juvenile rhesus monkeys who had been raised under conditions of mastery over appetitive non-aversive stimuli were compared to controls raised in identical social cages except that the same stimuli (toys, food treats) were presented in a "yoked", non-contingent fashion. The repeated experience of mastery early in development led to less behavioral distress during isolation in young adulthood. Administration of the putative anxiogenic, B-CCE, was associated with robust but qualitatively different responses in the two groups. The "mastery" animals responded with aggression whereas animals in the yoked group became withdrawn, showing self-directed, "fearful" postures. These results shed light on one source of variance in the response to so-called "anxiogenic" drugs and they demonstrate that environmental conditions during development may contribute to lasting differences in benzodiazepine receptor mediated responses.

Section on Histopharmacology

David M. Jacobowitz, Ph.D., Chief

In the past decade, the Section on Histopharmacology has been involved with studies concerning the discrete localization of neurochemcials within the brain. Complete brain maps of monoaminergic and cholinergic nerves and eight neuropeptides have been accomplished. This major "depository" of information concerning sites of localization for potent brain neurochemicals has given birth to a field of neuroscience described by Dr. Jacobowitz as "brain cartography." It is the mapping of potential brain neuroregulators that serves as a springboard of ideas from which behavioral studies emanate. More recently, complete mappings of the localization of brain peptide receptors, as revealed by autoradiography, has been undertaken.

For the last five years a parallel program has been developing that concerns the mapping of brain proteins. The aim of this program is to reveal unusual proteins contained in various discrete nuclei and regions of the brain. We are currently generating antisera from such proteins which can be used as immunological probes, recognizing particular types of neurons, synapses or other structures. Such cytochemical markers have great potential for identifying cell classes and neuronal circuits. At this time five purified proteins have been injected into rabbits and we hope to be able to report the localization of novel neuronal populations using proteins as specific brain markers.

Using two-dimensional gel electrophoresis (2DE), we have constructed an atlas showing the location and relative concentration of a number of different proteins from 25 distinct neuroanatomical regions of the rat brain. The proteins are stained with silver and quantified by computerized densitometry. This atlas is a first attempt at systematically classifying the molecular weight, charge (pI) and relative concentration of proteins present in a variety of regions of the brain. A major strength of this method lies in its capacity to display a large number of proteins simultaneously, using just a few milligrams of tissue.

The subcellular distribution of proteins (cytosol, synaptic membrane, microsomes, mitochondria, nucleus, crude synaptic vesicles, myelin and synaptic membrane) normally visible on 2DE gels of rat brain were identified. This information should prove useful in future experiments designed toward isolating and characterizing specific proteins of neurochemical interest. We have also identified calmodulin-binding proteins and are currently preparing to identify the calcium-binding proteins on the gels.

The identity of most of the protein spots demonstrated by 2DE has yet to be established. Using immunoblotting and comigration techniques, we have identified major proteins seen on normal rat and human cerebral cortex and of certain human brain tumors. These proteins include: actin, albumin, alpha-tubulin, beta-tubulin, neuron specific enolase, non-neuronal enolase, catechol-o-methyl transferase (COMT), glutamate oxaloacetic transaminase (sGOT), tyrosine hydroxylase, glial fibrillary acidic protein (GFAP), vimentin, a 66 kD intermediate filament protein, the 68 kD neurofilament protein, calcineurin, S-100 protein and the beta-subunit of the guanine nucleotide regulatory proteins (G-proteins). While many proteins remain to be identified on 2DE gels, certain important strides have been made in this direction. The information contained in this report will provide other workers in the field with a basis for reference and comparison. This is likely to accelerate the process of protein identification on 2DE gels and to enhance the value to this technique in the study of CNS proteins.

Previous studies have shown that the disruption of the cholinergic input to cortex and hippocampus results in an alteration in the concentration of a limited number of proteins in both of these brain areas. In contrast, destruction of the noradrenergic system in brain by either an electrolytic lesion of the locus coeruleus or neonatal administration of 6-hydroxydopamine produces a much more complex pattern of protein changes, with different brain areas exhibiting changes in different proteins. More recently we have identified proteins which are apparently regulated in concentration in two different areas of the rat brain by the indole neurotransmitter serotonin. Reduction in brain serotonin (5HT) levels following intraventricular injection of the 5HT neurotoxin, 5,7-dihydroxytryptamine produced a decrease in the concentration of three different proteins (two in the parietal cortex, one in the hippocampus). Two proteins, both in the hippocampus, were elevated in concentration following 5HT depletion. One protein (#102) found to be reduced in concentration in the hippocampus of 5HT-depleted rats, was identical to 6-hydroxydopamine-pretreated rats (norepinephrine depleted). This suggests that protein #102 in the hippocampus may be regulated by both norepinephrine and 5HT. This is of interest in light of recent studies which have indicated that there may exist a reciprocal functional relationship between certain noradrenergic and serotonergic axons in the CNS (Heydorn).

It has recently been shown that the arcuate nucleus of the hypothalamus undergoes major structural changes during normal puberty or if exposed to a pulse of estradiol in the prepuberal period. Those changes are expressed by increased synaptogenesis and by a drastic alteration in the feedback control of anterior pituitary gland hormone release. We have observed that the administration of estradiol to 25-day-old female rats resulted in changes in protein concentration of the arcuate nucleus-median eminence complex. Out of 235 proteins measured, eight proteins were found to be significantly increased and four were decreased by estrogen treatment. In a second experiment the brains of similar estrogen-treated rats were cut fresh on a Vibratome and incubated in a medium containing ^{35}S-methionine and ^{35}S-cyteine. The incorporation of labeled amino acids was increased in 10 proteins of rats killed at 17 hours after estrogen, while three proteins decreased. At 42 hours after estrogen, six proteins showed an increased incorporation of labeled amino acids and two proteins showed a decrease. These results focus attention on a group of proteins that respond to estrogen treatment during the puberal period of development in the female rat. It is possible that one or more of these proteins may be a synaptic growth factor induced by estradiol (Rodriguez-Sierra).

Guanine nucleotide regulatory proteins (G proteins) are a family of membrane-bound polypeptides involved in signal transduction. We have studied the localization of the beta-subunit of the G protein. Our results demonstrate that the beta-subunit of the G protein from both bovine rod outer segment membranes (transducin) and from bovine brain exists as multiple charge isomers. Using two-dimensional gel electrophoresis, the purified G protein beta-subunit can be shown to exist primarily as a pI 6.0 protein with minor species being detected at pI 5.8 and 5.5. Two different molecular weight forms (36 kD and 34 kD) of each charge isomer were also noted, with the 36 kD protein being the primary form detected. Immunoblot studies demonstrate that all charge and molecular weight forms of the G protein beta-subunit are recognized by antisera raised against both transducin and the bovine brain G proteins, further suggesting an immunological homology of this protein. Both rat and human brain apparently contain only the pI 5.8 isomer of this protein. Semiquantitative analysis of the regional distribution of the G protein beta-subunit in rat brain reveals that, in general, cortical areas contain the largest amount of this protein. Finally, the subcellular distribution of the G protein beta-subunit shows that most of this protein can be found in the membrane-derived fractions, with the smallest amounts detected in the cytosolic and mitochondrial fractions (Heydorn).

Studies investigating the effects of iron deficiency have demonstrated that cognition as well as learning deficits occur in this disorder. Recent studies examining learning and memory have begun to focus on specific changes in brain proteins to account for these processes. Using the technique of two-dimensional gel electrophoresis, we wanted to begin to address the issue of whether protein changes in the brain could account for deficits in cognition and learning that occur subsequent to iron deficiency. The present study revealed that ten different proteins in the rat nucleus accumbens and caudate nucleus were altered in iron-deficient rats. In the caudate nucleus, the relative concentrations of three proteins were significantly increased, whereas the relative concentration of one protein was significantly reduced by iron deficiency. In the nucleus accumbens, the relative concentration of three proteins were significantly increased by iron deficiency. In contrast, four proteins were significantly reduced in relative concentration by iron deficiency. One protein, albumin, was found to be significantly elevated in both brain regions and is thought to reflect an increase in local blood flow. The identity of two other proteins that were altered by iron deficiency is also known. Neuron-specific enolase was significantly elevated in the nucleus accumbens in iron-deficient rats, and glial fibrillary acidic protein was significantly reduced in the caudate nucleus. Interestingly, one of the proteins found to be reduced in the nucleus accumbens has the same molecular weight as the dopamine D_2 receptor, which has also been shown to be reduced by iron deficiency. Further study into the relevance of these protein changes will be necessary before it is known if these proteins are involved in cognition and learning (Sills; Youdim).

Our program on brain neuropeptides involves the immunocytochemical localization, radioimmunoassay quantitation, HPLC identification and behavioral analysis. We have accomplished these studies with the atrial natriuretic peptide (ANP). ANP was originally shown to be present in the atrial muscle of animals and not in nerves. This is a physiologically remarkable peptide in that it is a hypotensive compound that is also a potent natriuretic and diuretic. Because of its potential clinical usefulness it has generated enormous interest in the academic and pharmaceutical communities. We have shown for the first time that ANP has a widespread distribution in the brain. The distribution of immunoreactive (ir) atrial natriuretic peptides (ANPs) in 47 microdissected brain and spinal cord regions of the rat was determined by radioimmunoassay. The highest concentrations of ir-ANPs exist in the paraventricular nucleus and median preoptic nucleus. High concentrations of ir-ANP are present in the interpeduncular nucleus, preoptic and hypothalamic periventricular nuclei, median eminence and organum vasculosum of the lamina terminalis. Moderate concentrations of ir-ANPs were found in 16 brain regions such as the bed nucleus of the stria terminalis, nucleus of the diagonal band, most of the hypothalamic nuclei, central gray, locus coeruleus and parabrachial nuclei. Low levels of ir-ANPs exist in 22 rat brain regions including cortical areas, amygdala, caudate nucleus, nucleus accumbens, hippocampus, supraoptic nucleus, subfornical organ, medial mammillary nucleus, substantia nigra, dorsal raphe nucleus, cerebellum, nucleus of the solitary tract and others. Cervical spinal cord and neurointermediate lobe of pituitary gland contain low levels of ir-ANPs. We have also demonstrated that pmol quantities of ANP injected into the AV3V area of the anesthetized rat produces substantial increases in both blood pressure and heart rate. It therefore appears that ANP may play a central cardiovascular regulatory role (Skofitsch, Sills, Zamir).

We have recently mapped a comparatively new peptide, galanin, and have found it to be widely distributed in the rat central nervous. Interestingly, all cells in the locus coeruleus contain immunoreactive galanin and therefore this peptide coexists with norepinephrine-containing nerves that project to the cortex, hippocampus, thalamus, parts of the hypothalamus and spinal cord. Radioimmunoassay of discrete areas of the brain revealed high concentration of galanin in the amygdaloid nuclei, the septum, globus pallidus, bed nucleus of the stria terminalis, all hypothalamic nuclei, the superior colliculus, locus coeruleus, the nucleus of the solitary tract and the neurointermediate lobe of the pituitary. Autoradiographic mapping of ^{125}I-galanin receptor binding sites was accomplished. Dense binding was observed in the prefrontal cortex, the anterior nuclei of the olfactory bulb, several nuclei of the amygdaloid complex, the dorsal septal area, dorsal bed nucleus of the stria terminalis, the ventral pallidum, the internal medullary laminae of the thalamus, medial pretectal nucleus, nucleus of the medial optic tract, borderline areas of the caudal spinal trigeminal nucleus adjacent to the spinal trigeminal tract, the substantia gelatinosa and the superficial layers of the dorsal spinal cord. The preponderance of galanin-binding in somatosensory as well as limbic areas suggest a possible involvement of this peptide in a variety of brain functions (Skofitsch).

Annual Report for the Child Psychiatry Branch
National Institute of Mental Health
October 1, 1985 - September 30, 1986
Judith L. Rapoport, M.D., Chief

The Child Psychiatry Branch, formed in October 1984, conducts research on biological aspects of child psychiatry. Newer neuroradiological techniques and response to pharmacologic agents are current tools for this research. A great deal of effort goes into developing methodology for clinical research with children and adolescents. This has included standardization of activity monitoring, ward rating scales and attentional tasks for hyperactive children, self report measures for obsessive compulsive children, and reading and spatial tests for dyslexic subjects during cerebral blood flow measurement.

There are several major collaborators with this program. Within the NIMH, there are ongoing collaborations with the LPP (Drs. Zahn, Duncan-Johnson & Coppola), the LCS (Drs. Potter, Medford), the NPB (Drs. Weinberger & Berman) and LCM (Dr. R. Cohen). Collaborations are also taking place with other NIH institutes: NINCDS (Drs. Martha Denckla, Drs. Paul Fedio and Chris Cox, NIAAA (Dr. Linnoila), NIA (Dr. Stanley Rapoport & staff) and NEI (Dr. Goldberg).

Finally, collaboration with other institutions are: Dr. Agnes Whitaker (Columbia University School of Medicine), Dr. Eric Taylor (The Maudsley Hospital, London), and Dr. James Swanson (University of California, Irvine).

The Child Psychiatry Branch is focusing on three broad areas of clinical pathology. Because of the very heavy association between behavioral disturbance and developmental disabilities, approximately one third of Branch resources are devoted to studies of pervasive and specific developmental disorders such as autism or specific learning disabilities. Initial brain-mapping studies of adults with severe dyslexia have been completed, utilizing the xenon inhalation technique. A differential pattern of stimulation for dyslexics is suggested with relatively greater right sided increase in cerebral blood flow for the dyslexics in response to verbal stimuli. A pilot study with MRI scanning indicates lack of normal asymmetry of the occupital lobes for the dyslexic group.

Studies of childhood Obsessive Compulsive Disorder indicate far greater prevalence (about .7%) in unselected adolescent populations than had been previously estimated. Subjects with childhood onset are males, often from psychiatrically normal, families and associated movement disorders and neuropsychological test patterns suggest CNS insult probably of the basal ganglia.

ANNUAL REPORT OF THE CLINICAL NEUROSCIENCE BRANCH
National Institute of Mental Health
October 1, 1985 - September 30, 1986

Steven M. Paul, M.D., Chief

Introduction

The Clinical Neuroscience Branch conducts an interdisciplinary research program in the neurosciences with a major emphasis on elucidating the basic brain mechanism(s) involved in the etiology, pathophysiology, and treatment of the major neuropsychiatric disorders. The Branch consists of four sections, 1) the Section on Preclinical Studies (Steven M. Paul, M.D., Chief), 2) the Section on Brain Biochemistry, (Candace B. Pert, Ph.D., Chief), 3) the Section on Molecular Pharmacology (Steven M. Paul, M.D., Acting Chief), and 4) the Section on Clinical Studies (David Pickar, M.D., Chief). In addition, three units have been formed in the Sections on Molecular Pharmacology and Preclinical Studies: 1) the Unit on Behavioral Pharmacology (Jacqueline N. Crawley, Ph.D., Chief); 2) the Unit on Histochemistry and Electrophysiology (Drs. Daniel Hommer, M.D., and Lana Skirboll, Ph.D.; Chief); and 3) the Unit on Molecular Neurogenetics (Dr. Edward I. Ginns, M.D.; Chief). The current research focus of the members of the Branch are quite diverse and although an attempt is made to emphasize studies related to the basic pathological (or pathophysiological) processes underlying neuropsychiatric disease, individual investigators are encouraged to work on any problem related to the basic mechanisms of brain function. In general, the major areas of investigation involve characterizing critical aspects of synaptic transmission including the various neurotransmitter and neuromodulator substances themselves as well as their biosynthetic and metabolic enzymes.

With the recruitment of Drs. Edward Ginns and Brian Martin, we have initiated a major commitment to protein biochemistry, sequencing, and molecular neurogenetics. A number of collaborative efforts are underway which involve the use of recombinant DNA methods to study the genes for various membrane proteins, including ion channels and receptors. A brief summary of the various research projects and some of the results to date, follows.

SECTION ON PRECLINICAL STUDIES

Steven M. Paul, M.D., Chief

Members of the Section on Preclinical Studies continue to study the mechanisms of action of important psychotropic agents. The principal drugs under investigation include the minor tranquilizers, (i.e. benzodiazepines and barbiturates) as well as the tricyclic antidepressants, and various psychomotor stimulants, including amphetamine and methylphenidate. Work on the benzodiazepine/GABA receptor complex has continued with a primary emphasis on studies measuring GABA-stimulated chloride (Cl^-) ion flux in a subcellular brain preparation, the synaptoneurosome. Over the past two years we have succeeded in developing a reliable method for measuring GABA receptor and barbiturate receptor-mediated Cl^- efflux and uptake in this subcellular brain preparation.

A series of experiments has established the validity of this method for measuring the functional activity of the GABA receptor-gated ion channel.

During this past year a number of experiments on the "sensitization" and "desensitization" of this receptor complex have been carried out and these studies have resulted in several publications describing desensitization and cross-desensitization of this receptor complex induced by various agonists. Using both radioligand binding methods and flux measurements we have also shown that "stress" is a major sensitizer of the GABA-gated chloride ion channel, inducing a prompt increase in the ability of GABA to stimulate chloride ion flux. Corresponding changes in ligand binding to the receptor complex including increases in the specific binding of [^{35}S]-t-butylbicyclophosphorothionate (TBPS), and the anion sensitivity of benzodiazepine agonist binding have also been demonstrated in the same stress paradigms. The mechanisms underlying the rapid "sensitization" and desensitization of the GABA receptor complex are still unknown and are currently being pursued using this in vitro method. To date a number of hormones including the mineralcorticoid metabolite tetrahydrodeoxycorticosterone, and the glucocorticoid, corticosterone, have been shown to regulate the "sensitivity" of the GABA-gated chloride ion channel. These effects, we believe may be important in explaining the changes in this receptor that occur following exposure to environmental "stressors" in animals. It is conceivable that similar effects occur in man, and that these may underlie a physiologically-relevant adaptation to stress.

Over the past year Dr. Peter Suzdak has continued his studies on the interaction of ethanol, and other short-chain alcohols, on the GABA-gated Cl^- ion channel. Dr. Suzdak's initial studies demonstrated that ethanol and all short-chain alcohols stimulate GABA receptor mediated Cl^- uptake and at concentrations that are pharmacologically relevant (i.e. those observed during acute intoxications). The effects of these alcohols in stimulating GABA receptor mediated Cl^- uptake were highly correlated with their intoxication potencies in animals, and their membrane buffer partition coefficients; suggesting that these alcohols were working by disturbing the microenvironment of the receptor complex rather than directly binding to receptor protein. In very recent studies, Dr. Suzdak has found a novel imidazobenzodiazepine (Ro15-4513) which is an azido derivative of the benzodiazepine receptor antagonist Ro15-1788 that selectively blocks the ability of ethanol to stimulate chloride ion uptake in vitro and also prevents and reverses many of the behavioral actions of ethanol in vivo. The results with this imidazobenzodiazepine compound support our hypothesis that many of the neuropharmacological actions of ethanol are related to an augmentation of GABA receptor-mediated neuronal events. The discovery of a selective antagonist of ethanol in animals suggests that an alcohol antagonist could be developed for clinical purposes and may be useful in the treatment of chronic alcoholism.

In another series of studies we have explored the interaction of naturally-occurring steroids with the GABA/benzodiazepine receptor complex. These studies are an extension of Dr. Dorota Majewska's work which has defined the existence of two naturally-occurring steroid hormones, the metabolite of progesterone (3α,5α-dihydroprogesterone) and deoxycorticosterone (3α-tetrahydrodeoxycorticosterone) which are relatively potent ligands in binding to the GABA receptor chloride ion channel complex and behave in a similar fashion to barbiturates in potentiating the effects of GABA at this complex. These data along with behavioral work done by Drs. Crawley and Glowa suggest that these naturally-occurring ring A reduced steroids act as barbiturate-like ligands of the GABA receptor complex and may be released during

stress to modulate GABA mediated synaptic events. Other studies have focused on the regulation of glutamic acid decarboxylase (GAD) messenger RNA transcription using a recently cloned cDNA probe for GAD. Drs. Yong Sik Kim, Peter Suzdak, and John Thomas have been studying the levels of GAD mRNA following a variety of pharmacological and physiological conditions. The use of this probe may enable us to study dynamic changes in the GABA system and preliminary results suggest a feedback regulation of GAD synthesis by intrasynaptic GABA concentration.
In the past year we have also continued our studies on the anorectic drug recognition sites in brain labelled with [^{3}H] (+)-amphetamine and [^{3}H] mazindol. Dr. Itzchak Angel, who recently left the laboratory, to direct his own laboratory in a private pharmaceutical company in Paris, had succeeded in labelling these anorectic drug recognition sites with [^{3}H] mazindol and had shown that the characteristics of the [^{3}H] mazindol binding site were virtually identical to those of the [^{3}H] amphetamine binding site previously demonstrated in our laboratory). Dr. Angel has extended his studies on the regulation of these sites _in vitro_ and _in vivo_ by glucose and ATP. In addition, Dr. Angel has shown that the regulation of these sites is related to changes in Na^+ K^+ ATPase activity and that this system appears to be dysfunctional in genetically obese (ob/ob) mice. In the latter experiments, Dr. Angel has shown that the genetically obese mouse has elevated levels of anorectic drug recognition sites and [^{3}H] ouabain binding sites (Na^+ K^+ ATPase activity) in hypothalamic membranes and that these sites are not further stimulated by incubation or administration of glucose. Studies are still underway to identify the exact protein labelled by these anorectic drugs.

Unit on Molecular Neurogenetics

Edward I. Ginns, M.D., Ph.D., Chief

The Molecular Neurogenetics Unit initiative is part of our effort to better understand the role of specific proteins and their genes in the molecular mechanisms underlying human nervous system development and function, particularly in relation to the pathogenesis of neurologic and psychiatric disorders. Th description of the synthesis, maturation, intracellular compartmentalization, and degradation of proteins, has been complemented by information obtained using recombinant DNA techniques to investigate gene organization. Our studies have focused on peptides and proteins that interact with excitable membranes (phospholipases and venom toxins), isozymes that are developmentally regulated within specific cell populations (neuron-specific and non-neuronal enolases) and enzyme deficiencies responsible for lysosomal storage disorders (glucocerebrosidase and alpha-galactosidase deficiency in Gaucher disease and Fabry disease, respectively).

The isolation of cDNA and genomic DNA for enzymes involved in the pathogenesis of several neurogenetic disorders (in particular Gaucher and Fabry diseases) have been an important step in the development of potentially useful recombinant DNA approaches to somatic cell gene therapy. Our successful correction of the enzyme deficiency in Gaucher cells in culture using retroviral-mediated somatic transfer of the human glucocerebrosidase gene into mice by bone marrow transplantation.

SECTION ON CLINICAL STUDIES

David Pickar, M.D., Chief

A major component of our research program concerns the neurobiology of schizophrenia. We have previously reported that administration of the neuroleptic, fluphenazine, produces significant time-dependent decreases and increases, respectively, in plasma levels of the dopamine metabolite, homovanillic acid (HVA) and have found that these changes are correlated with clinical response. We have continued to investigate the potential use of levels of plasma HVA as a marker for the antipsychotic response of neuroleptic treatment. Towards this end we have initiated studies using debrisoquin, a peripheral MAO inhibitor, which increases the relative central nervous system contribution to circulating levels of plasma HVA. Preliminary data from debrisoquin-treated subjects also show a time-dependent decrease in plasma levels of HVA suggesting that the pattern of response previously observed may parallel CNS dopamine system changes.

Two controlled drug treatment trials were performed in schizophrenic patients. The calcium channel antagonist, verapamil, was observed to lack therapeutic effects in otherwise drug-free schizophrenic patients. We did observe, however, that the addition of the triazolobenzodiazepine, alprazolam, to a stable neuroleptic treatment regimen produced significant group improvement in 12 schizophrenic patients. This latter finding may have important clinical implications and provide new leads in understanding the pathophysiology of schizophrenia. Structural brain abnormalities in schizophrenic patients have been studied by applying CT methodologies to medical patients, normal controls and schizophrenic patients. Our findings suggest that prefrontal cortical atrophy, but not lateral ventricular enlargement nor generalized atrophy, is a unique structural abnormality found in schizophrenia. These finding add to expanding data implicating frontal cortical dysfunction in schizophrenia.

The Section has also continued its program in the study of affective disorders. Dr. Owen M. Wolkowitz has initiated important work examining the effect of corticosteroids on the CNS. Studies have included the effect of dexamethasone on levels of catecholamine metabolites and the prospective administration of oral prednisone (80 mg/day x 5 days) to normal controls. Data from these experiments are consistent with the notions of disordered steroid-catecholamine relationship in depression and that the hypercortisolemia itself may produce behavioral and biochemical signs of depression. In other work, Dr. Alan Breier has developed a unique paradigm in which the behavioral and biological response to "escapable" versus "nonescapable" aversive "stress" in humans is studied. Preliminary data has demonstrated that nonescapable in comparison to escapable aversive stimuli produces increased ratings of stress, depression and anxiety and stimulation of HPA axis. This project is a potential "model" for depression in normal subjects and will be used as a probe for studying biological diatheses in depression and in subjects at risk for depression.

Drs. Rex Cowdry and David Gardner have extended their studies of borderline personality disorder, focusing on biological correlates of mood lability and behavioral dyscontrol. The high incidence of "soft" neurological signs and the regular induction of severe dysphoria by procaine infusion have been important observations. Pharmacologic challenges which may provoke dysphoric states in borderline personality subjects are being evaluated as biological probes for this illness.

Dr. Daniel Hommer, M.D., has utilized the strategy of diazepam infusions in which saccadic eye velocity is determined as a measure of benzodiazepine receptor sensitivity. Studies have included pretreatment with the benzodiazepine antagonist, Ro15-1788, and the determination of receptor sensitivities of different diagnostic groups of psychiatric patients.

SECTION ON MOLECULAR PHARMACOLOGY

Steven M. Paul, M.D., Acting Chief

The Section on Molecular Pharmacology consists of two independent units, the Unit on Histochemistry and Neurophysiology, (Drs. Lana Skirboll and Daniel Hommer, Chiefs) and the Unit on Behavioral Pharmacology (Dr. Jacqueline N. Crawley, Chief).

Unit on Histochemistry and Neurophysiology

Drs. Lana Skirboll, Ph.D. and Daniel Hommer, M.D.; Chief

Dr. Skirboll and colleagues have continued immunohistochemical studies combined with retrograde tracing to examine the innervation of several brainstem nuclei. In several brainstem nuclei it is apparent that more than one neurotransmitter coexists in the same cell body. Dr. Skirboll's work has defined the various neurotransmitter substances in a single cell body and she has begun to ascertain the physiological significance of coexistence in brain. In other studies, Dr. Skirboll and colleagues have been studying the paraventricular nucleus (PVN) of the hypothalamus and especially with respect to the innervating transmitters present in the afferents to this area. Dr. Skirboll has confirmed using retrograde staining with fluorescent dyes that the epinephrine-containing cells of the PVN originate in the C1 and C2 areas of the medulla oblongata. These studies are important in that they define the various neurotransmitter candidates that are involved in the release of CRF and in the regulation of the hypothalamic pituitary adrenal axis. In a recent series of experiments, Dr. Skirboll has been exploring the possibility that dynorphin and GABA coexist in the same neuron in a projection from the caudate nucleus to the substantia nigra zona reticulata. The descending pathway from the striatum to the substantia nigra has been well known to be an important regulator of dopamine-containing cell bodies in the substantia nigra and recent evidence from electrophysiological experiments suggest that dynorphin iontophoretically applied to nigral neurons may alter the inhibitory action of GABA itself.

Drs. Hommer and Skirboll along with Drs. Robert Drugan and Jacqueline Crawley have continued their studies on the effects of "stress" on the sensitivity of GABA receptors, localized to the substantia nigra zona reticulata. It appears that subjecting animals to inescapable aversive tailshock results in a marked supersensitive response to intravenously administered muscimol and to iontophoretically applied GABA. Animals subjected to an identical shock exposure (but with control over terminating shock) did not develop this supersensitive response to muscimol. Thus, uncontrollable stress may lead to an increased sensitivity of GABA receptors localized on the substantia nigra, zona reticulata neurons. Studies using iontophoretically administered GABA are now in progress to substantiate a direct effect on the GABA receptor itself. These studies are among the first to attempt to study the electrophysiological consequences of uncontrollable versus controllable stress. Drs. Skirboll and Hommer have continued their work on cholecystokinin (CCK)-dopamine interactions in the substantia nigra. They have extended their previous results demonstrating that sulfated CCK-8 potentiate the effects of the dopamine agonist, apomorphine on DA neurons in the medial substantia nigra. More recently they have found that unsulfated CCK-8 and CCK-4 possess a similar ability to potentiate apomorphine induced inhibition in this area. The relative potencies of various peptide fragments in potentiating apomorphine induced inhibition correlated very well with their affinity for brain CCK receptors. In contrast, only CCK-8 sulfate produced an excitatory effect on DA neurons, suggesting a relationship with the peripheral type of CCK receptor. Thus, Drs. Hommer and Skirboll have confirmed the functional significance of the brain-specific CCK receptor as characterized by radioligand binding studies.

Unit on Behavioral Neuropharmacology

Jacqueline N. Crawley, Ph.D., Chief

Dr. Crawley and her collaborators continue to explore the functional significance of peptides coexisting in the same neuron as "classical" transmitters and have focused on the role of endogenous peptides in the awake, behaving rat. To study the coexistence of cholecystokinin and dopamine, a paradigm of electrical stimulation of the ventral tegmentum is under development to activate the mesolimbic dopamine pathway and induce hyperlocomotion non-pharmacologically. A cholecystokinin antagonist, proglumide, as well as a dopamine antagonist, haloperidol, blocked VTA-stimulated hyperlocomotion, suggesting that endogenous CCK plays a major role in activating postsynaptic neurons of the mesolimbic dopamine pathway. Using carbachol-induced "boxing"-like seizures as a model to study the triple coexistence of acetylcholine, substance P and corticotropin releasing factor in the prefrontal cortex of the rat, Dr. Crawley and colleagues have delineated both anatomical and peptide specificity to the modulatory effects of SP and CRF on carbachol-induced "boxing." Jill Stivers found that SP antagonists significantly attenuated carbachol-induced "boxing," suggesting that endogenous SP plays a role in facilitating cholinergically-induced seizures. A third case of coexistence is under investigation, the case of two peptides with no "classical" transmitter, i.e. oxytocin coexisting with cholecystokinin in the supraoptic and paraventricular nuclei of the hypothalamus. Maria Kaltwasser, a postdoctoral fellow, found that in this case, both peptides were active in inducing grooming behaviors, as opposed to the modulatory activity of neuropeptides coexisting with "classical" transmitters. These peptide studies may lead to the development of analogs and antagonists of neuropeptides for the treatment of disorders such as schizophrenia and epilepsy.

Animal models of neuropsychiatric disorders have been extensively studied and include the dwarf hamster separation model of depression, the learned helplessness syndrome of depression, mouse exploration and rat thirsty-lick models of anxiety, 2-deoxyglucose and starvation models of feeding disorders, and the rating scale for ethanol-induced intoxication. Dr. Crawley found that an endogenous mineralocorticoid metabolite of progesterone, THDOC, identified by Majewska, Schwartz, and Paul as acting through the central benzodiazepine-GABA-chloride channel receptor complex, acts as an anxiolytic in vivo. Robert Drugan, a postdoctoral fellow, has shown that the learned helplessness paradigm alters [^{35}S] TBPS binding to the benzodiazepine-GABA-chloride channel receptor complex. Dr. Drugan has also refined the learned helplessness paradigm to distinguish a pure population of rats developing the syndrome from non-syndrome rats, for increased accuracy in biochemical studies. An imidazobenzodiazepine discovered by Suzdak and Paul which blocks the biochemical actions of ethanol in biochemical studies of chloride flux was tested by Drs. Suzdak and Crawley and found to both prevent and reverse the behavioral manifestations of ethanol-induced intoxication in the rat.

SECTION ON BRAIN BIOCHEMISTRY

Candace B. Pert, Ph.D., Chief

Members of the Section on Brain Biochemistry have continued and extended their studies on the shared neurotransmitter/neuromodulator receptors that are common to both neurons and cells of the immune system. Drs. Pert and Ruff have been using the human monocyte model extensively to study signal peptide induced chemotaxis and have demonstrated that a number of neuropeptides including those thought to be important neurotransmitter substances including beta-endorphin, substance P, bombesin, and cholecystokinin stimulate chemotaxis. More recently, Dr. Pert and her collaborators have been studying the differentiation antigen T4, which is present on the helper/inducer subset of T lymphocytes, and is believed to be a receptor for the HIV virus. Dr. Pert's group finds that a 60 Kd protein immunoprecipitable by a monoclonal antibody to OKT4 is present on membranes from human brain as well as T lymphocytes. Furthermore, the HIV envelope glycoprotein can be specfically and covalently affixed to a molecule present on rat, monkey, and human brain membranes to yield a complex which is indistinguishable from that found on human T cells. T4 antigen has been studied in unfixed squirrel monkey, rat and human brain sections by autoradiography using the monoclonal antibody to OKT4. A highly conserved neuroanatomical pattern has been demonstrated suggesting an analogous organization in these three mammalian brains. Furthermore, the localization of ^{125}I-gp120 receptor binding appears similar to that of T4 and is highly reminiscent of patterns for many previously characterized neuropeptide receptors.

Using a computer-assisted analysis of the viral envelope glycoprotein (gp120) Dr. Pert and her collaborators have observed previously unremarkable octapeptide sequences within the gp120 protein, which have been synthesized and termed "peptide T". Dr. Pert and her colleagues believe this sequence may play an important role in HIV attachment. In related studies, peptide T and 3 rationally designed peptide analogs each with a systematic amino acid substitution have been shown to potently inhibit specific ^{125}I-gp120 binding to brain membranes. Additionally, when tested in a viral infectivity assays these peptides show the same rank order and similar absolute potencies in blocking HIV infection of human T cells. These studies suggest that peptide T may provide a useful pharmacological or immunological bases for the control and treatment of Acquired Immunodeficiency Syndrome (AIDS).

Dr. Pert's group has also continued their investigation on the characterization of opiate receptors in various species. In collaboration with Dr. Birgit Zipser, she has succeeded in cross linking the opiate receptor with an iodinated derivative of beta-endorphin. These studies have shown remarkable conservation of the opiate receptor among a whole host of species including tetrahymena, leech, rat, and human. Dr. Pert and her group are also continuing their work on the use of fluorinated opiate derivatives for use in visualizing the opiate receptor using positron emission tomography. The opiate probe 3-acetylcyclofoxy has now been studied in monkeys and it is anticipated that clinical studies with this ligand should commence shortly. Finally, Drs. Pert and Knight are continuing their studies on the presence of antibrain antibodies in the sera of schizophrenic patients and in testing the autoimmune hypothesis of schizophrenia. To date several new methods have been set up for detecting the presence of these antibrain antibodies using a variety of different approaches. It is hoped that using these screening methods such antibodies will either be detected or their absence will be firmly documented.

Annual Report of the Laboratory of Neuropsychology

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Mortimer Mishkin, Ph.D., Chief

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Two cortical visual pathways

Cortical tissue essential for visual perception extends far beyond the primary visual area, striate cortex, to include not only the prestriate regions of the occipital lobe but also large portions of the temporal and parietal lobes. Converging evidence from our neurobehavioral and neurobiological studies indicates that these extrastriate regions contain numerous areas that can be distinguished both structurally and functionally. Moreover, the multiple visual areas appear to be organized hierarchically into two separate cortical visual pathways, each having the striate cortex as the source of its initial input.

The first pathway consists of an occipitotemporal projection system. This pathway, which courses ventrally to interconnect the striate, prestriate, and inferior temporal areas, is crucial for the visual recognition of objects. Links between this occipitotemporal pathway and limbic structures in the temporal lobe as well as ventral portions of the prefrontal lobe appear to make possible the cognitive association of visual objects with other stimuli and other events, such as emotions and motor acts. The other pathway consists of an occipitoparietal projection system. This pathway, which courses dorsally to interconnect the striate, prestriate, and inferior parietal areas, is critical instead for the visual localization of objects. Links between the occipitoparietal pathway and both dorsal limbic and dorsal prefrontal cortex enable the cognitive construction of spatial maps, as well as the visual guidance of motor acts that may have been triggered initially by activity in the occipitotemporal pathway. In contrast to the occipitotemporal pathway, which remains modality-specific throughout its course, the later stations in the occipitoparietal pathway appear to receive convergent input from other modalities and so constitute polysensory areas.

To trace the flow of information through the two cortical visual pathways in detail, we have undertaken a series of anatomical studies using both anterograde and retrograde tracing techniques (e.g., amino-acid autoradiography, horseradish peroxidase histochemistry, and axonal transport of fluorescent dyes) in combination with electrophysiological recording. Our goal in these studies is to identify the multiple visual areas that comprise this cortex, delineate their topographic organization, and explore the complex circuitry of their interconnections.

Our results indicate that the occipitotemporal pathway begins with the striate projection to the second and third visual areas, V2 and V3, which in turn project to area V4. These three prestriate areas are arranged in adjacent belts that nearly surround the striate cortex, and, like the striate cortex, each belt contains a representation of the contralateral visual field. Area V2 corresponds to cytoarchitectonic area OB, while V3 and V4 together correspond to area OA, exclusive of its dorsal part. The major output of V4 is to a widespread region within the inferior temporal cortex. Within posterior IT cortex, or architectonic area TEO, label was found only after V4 injections involving the representation of the central 10° of the visual field, whereas within anterior IT cortex, or architectonic area TE, label was found after injections of any part of V4. Thus, central but not peripheral field representations in V4 project to TEO, while both central and peripheral field representations in V4 project to TE. Physiological studies have shown that, unlike the visual areas that precede it, TE has no discernible visuotopic organization. Rather, neurons in TE have very large receptive fields that nearly always include the center of gaze and frequently cross the vertical meridian into the ipsilateral visual field. Interestingly, TE appears to receive a very complex pattern of projections from V4, with a single site in V4 projecting to several separate TE fields that interdigitate with the multiple projection fields of other sites in V4. Thus, there may be a mosaic of visual areas within the temporal lobe, and we are currently investigating this possibility. As described below, our results on the occipitoparietal pathway suggest that there may be a mosaic of visual areas within the parietal lobe also. Area TE appears to be the last exclusively visual area in the occipitotemporal pathway for object recognition, as its cortical outputs are to areas in the temporal and frontal lobes that are probably multimodal in function.

Although an occipitotemporal pathway consisting of multiple visual areas has now been demonstrated in a variety of monkey species, suggesting a common primate plan, this notion has not yet been applied to the visual system in man. Indeed, concepts regarding the organization of the human visual system have not progressed much since the turn of the century. For example, many complex visual syndromes in man, including visual agnosia, prosopagonosia, and loss of recent visual memory, are still being attributed in the clinical literature to lesions of the inferior longitudinal fasciculus (ILF), commonly considered to be a long association fiber bundle interconnecting the occipital and temporal lobes. A long fiber bundle of this type has never been seen in any of our axonal transport studies in the monkey, however; and, indeed, in blunt dissections of both human and monkey brains that we performed recently to reexamine this question, we found that the only long fiber bundle common to both the occipital and temporal lobes is the geniculostriate pathway. Our axonal transport experiments in the monkey indicate that the pathway from occipital to temporal cortex in the monkey consists of a series of U fibers which course beneath the cortical mantle to connect adjacent regions in striate, prestriate, and inferior temporal cortex. By implication, the occipital and temporal lobes in humans are similarly connected by a series of U fibers, and, therefore, the various clinical syndromes that are still being attributed to ILF lesions are probably due instead to interruption of these short U-fiber connections at different points along a multisynaptic occipitotemporal pathway. Further study of human brain tissue is being planned, with the goal of identifying architectonically the stations in the human visual system that are homologous to those that have now been identified in the monkey.

Our results indicate that, unlike the occipitotemporal pathway, the occipitoparietal pathway begins with striate, V2, and V3 projections to visual area MT, which is located in the caudal portion of the superior temporal sulcus, mainly within the dorsolateral portion of cytoarchitectonic area OA.

MT projects to three additional areas located in the intraparietal and superior temporal sulci, and these project in turn to the inferior parietal lobule, area PG. Thus, although MT receives inputs from areas that participate in the occipitotemporal pathway, its outputs appear to be directed mainly into the parietal lobe.

MT is known to be insensitive to both the form and color of a visual stimulus, but is highly sensitive to the direction of stimulus motion. Our physiological experiments along the occipitoparietal pathway indicate that MT and the areas to which it projects may constitute a cortical system for motion analysis. At each successive stage of this system, neurons integrate motion information over increasingly large portions of the visual field, respond selectively to more complex types of motion, and respond to inputs from additional sensory modalities. In addition, these areas may become increasingly involved in visuomotor control, inasmuch as lesions along the occipitoparietal pathway cause impairment not only in visuospatial perception but also in eye movements and in visually guided hand movements.

Although MT plays a pivotal role in the occipitoparietal pathway, it does not provide the sole route by which visual information from striate cortex reaches the parietal lobe. Other potential pathways include those through additional visual areas located in occipito-parietal cortex. Our recent findings indicate that these areas receive inputs representing predominantly the peripheral visual field, which presumably reflects the importance of such inputs for spatial vision. We have also found a predominance of peripheral over central field inputs in the projections of V4 to area TF on the parahippocampal gyrus, suggesting that this temporal lobe region, like the parietal cortex, may have chiefly visuospatial functions. By contrast, the predominance of central field inputs from V4 to TEO in the posterior portion of the inferior temporal cortex presumably reflects the importance of these inputs for object vision.

The occipitotemporal pathway and stimulus encoding

Anatomical delineation of the occipitotemporal visual pathway has opened the way to a study of the functional properties of its single neurons. In an attempt to decode the visual information that they carry, we have developed a new method over the past few years that identifies and quantifies the temporal patterns of a neurons's stimulus-evoked discharge. The key to this new approach is the viewing of individual neurons as communication channels so that techniques from statistics, signal processing, and information theory can be applied.

The experiments were carried out by presenting to the awake, behaving monkey visual stimuli constructed from basic signal elements while we recorded from single neurons in both the first and last stations of the occipitotemporal pathway. We found that the neurons in both stations, namely, areas V1 and TE, convey stimulus information in the temporal modulation of their spike trains. The features of the neuronal responses that encoded the stimulus were identified, and we found that they usually consisted of 3-6 simultaneous but independent temporal patterns of neuronal activity. The amount of information about the stimulus conveyed by these different patterns was nearly twice as great as that transmitted by a more usual measure of the neuronal response, the spike count itself.

On the basis of these results, we have proposed a new model of the function of visual system neurons - the multiplex-filter hypothesis. The hypothesis states that neurons respond to visual stimuli as though they contained several, simultaenously active, spatial-to-temporal transforming or filtering mechanisms. The filtering mechanisms are reflected in the independent activity patterns or temporal modulations of the neuronal spike train, which can thus be viewed as containing a multiplexed message about the stimulus. If this hypothesis provides a reasonable description of the response properties of visual system neurons, then analysis of their responses to a basic stimulus set should yield sufficiently accurate characterizations of their multiple filters to allow predictions to be made of their responses to any other visual stimulus. We have tested such predictions using data collected from complex cells of striate cortex, cells whose nonlinearities of response would be expected to complicate any quantitative response descriptions. The neuronal responses to sixteen basic visual stimuli at seven sets of luminances were used to characterize the properties of the hypothesized spatial-to-temporal filters in several complex cells. Simulations based on the multiplex-filter hypothesis was used to predict the responses to 44 other stimuli. The predictions matched the actual responses extremely well in terms of both the temporal modulation and the magnitude of the responses. Thus, in spite of the known nonlinearities of complex cells, implementation of the multiplex-filter hypothesis yielded accurate quantitative predictions of their discharge patterns to new stimuli.

Because our results show that the responses of single visual system neurons can be regarded as containing several, simultaneous, low-resolution descriptions of the visual stimulus, we suggest a new view of the functional role of single neurons. Rather than conveying information about optimal or preferred stimuli selectively, each neuron may be transmitting a multiplexed message about every stimulus that enters its receptive field. The addition of sufficient numbers of neurons should allow specification of the stimulus to any desired level of resolution within the system's capabilities. Thus, the criterion of the accuracy of stimulus representation becomes the amount of information transmitted rather than the appearance of the strongest response. This conceptualization of neuronal function precludes the need for any neuron to signal an optimal stimulus.

The occipitotemporal pathway and the analysis of color, contour, and shape

Now that we know the location, visuotopic organization, and connections of many of the extrastriate areas in the occipitotemporal pathway, we can trace the transformation of visual information through them at the single neuron level. We began our physiological analyses in area V4, which plays a crucial role in the relay of visual information into the temporal lobe. We found that, as in striate cortex, some neurons in V4 are sensitive to object contours and low spatial frequencies, whereas others are more sensitive to textures and high spatial frequencies. Thus, the data on V4 suggest that within each visual area of the occipitotemporal pathway, the contours of object surfaces and the textures of object surfaces may be processed by separate populations of neurons. Surface color also appears to be processed by neurons within each of these visual areas. The results argue against the prevalent view that each area of the occipitotemporal pathway processes a different aspect of an object separately, such as color in V4 and depth in V2, and suggest instead that the different features of an object are processed in parallel within each area. At the same time, the results are consistent with our anatomical evidence indicating that the occipitotemporal pathway is organized as a serial hierarchy.

A hierarchical model predicts that the product of visual processing will become progressively more complex at each successive stage in the pathway. So far, our results in V4 support this prediction. In addition to sensitivity to the contour, texture, and color of a stimulus, many V4 neurons respond to a stimulus only if it stands out from its background on the basis of a difference in color or form. This responsivity to stimulus differences in the receptive field is due to a unique receptive-field structure of the neurons in V4: a small excitatory receptive field surrounded by a large, silent, suppressive zone. The surround zone is silent in that stimulation of it alone is without effect, but it is also suppressive in that it has properties antagonistic to those of the excitatory field and, hence, can suppress the response to an excitatory-field stimulus if the surround is stimulated in the same way. Thus, V4 neurons may play a role in separating figure from ground, a fundamental requirement for object perception.

Recently, we discovered that the suppressive surrounds of V4 neuronal receptive fields extend far across the vertical midline into the opposite visual hemifield and that this suppression is eliminated by section of the corpus callosum. Thus, the commissural inputs to V4 appear to be largely suppressive, in contrast to the commissural inputs to the inferior temporal cortex, which are known to be excitatory. These physiological results provide an explanation for a puzzling fact, namely, that despite heavy interconnections across the callosum, prestriate neurons, unlike neurons in the inferior temporal cortex, have excitatory receptive fields that are contralateral only.

Our findings that the corpus callosum provides a pathway for conveying inhibitory influences between the prestriate cortices of the two hemispheres but excitatory influences between the two inferior temporal cortices also resolves a paradox that had arisen from our 2-deoxyglucose studies. The results of these metabolic experiments provided clear evidence of commissural activation of the inferior temporal cortex but, surprisingly, not of any part of the visual system posterior to it. If the commissural input to posterior visual cortex is largely inhibitory, however, the functional consequences might well not be reflected in glucose metabolism.

The occipitotemporal pathway and selective attention

Our retinas are constantly bombarded by a welter of shapes, colors, and textures. Since we are aware of only a small amount of this information at any one moment, most of it must be filtered out centrally. Yet, this filtering cannot easily be explained by the known properties of the visual system. At each successive stage along the pathway from the striate cortex into the temporal lobe there is an increase in receptive field size. Many different stimuli will typically fall within these large receptive fields, and thus, paradoxically, more rather than less information appears to be processed by single neurons at each successive stage. How then does the visual system limit processing of unwanted stimuli? The results of our single-neuron recording experiments in visual cortex of trained monkeys indicate that unwanted information is filtered from the receptive fields of neurons in extrastriate cortex as a result of selective attention.

We trained monkeys to maintain fixation on a target while performing a task that required them to attend selectively to stimuli presented at one visual field location. Irrelevant stimuli were simultaneously presented at a location outside the monkey's focus of attention. When stimuli at both the attended and ignored locations were simultaneously present within the receptive field of a cell in either area V4 or the inferior temporal cortex, we found that the cell responded to stimuli at the attended location. For example, if the cell was selective for red stimuli, it would respond well if a red stimulus appeared at an attended location but poorly or not at all if a red stimulus appeared at an ignored location even though it was still in the receptive field. Thus, we have shown for the first time that the processing of unwanted visual stimuli in extrastriate cortex can be blocked as a result of selective attention. Our most recent evidence indicates that attention can also be directed to a specific sensory dimension of a complex stimulus, such as its texture or shape, and that the neuronal processing of irrelevant information may thereby be reduced even further. We propose that it is these extrastriate neural mechanisms for selective attention that enable us to identify and remember the properties of a particular stimulus out of the many that may be acting on the retina at any given moment.

Not only do extrastriate neurons fail to respond to unattended stimuli, but also the magnitude of their response to attended stimuli depends on "how much" attention, or effort, the animal devotes to the stimuli. Extrastriate neuronal responses to a given stimulus are larger and more tighly tuned when the monkey is discriminating that stimulus from one that is very similar to it than when the monkey is discriminating it from one that is very different. This enhanced processing of stimuli during the difficult task does not appear to be due to nonspecific arousal because responses to irrelevant stimuli, presented concurrently with the attended stimuli in the difficult task, are not enhanced. Thus, the information-processing capacity of cortical neurons appears to depend not only on hard-wired mechanisms but also on cognitive state.

Although we have found that the inputs to cells in V4 and the inferior temporal cortex are gated by attention, we have not yet identified the source of the gate. Our anatomical experiments on the occipitotemporal pathway indicate that the major source of visual inputs to V4 is area V2, yet we have not found any effects of attention in the latter area or in the primary visual cortex. The same anatomical studies suggest, however, that either the pulvinar, the posterior parietal cortex, portions of the prefrontal cortex, or the limbic system could be the source of the attentional gating process. Since we have recently ruled out the pulvinar on the basis of recording studies, our efforts over the next year will be focused on the remaining three anatomical possibilities.

A visual cortico-limbic circuit and recognition memory

Monkeys that are shown a series of objects once will demonstrate that they recognize them as familiar several minutes later by consistently choosing them over novel objects or by avoiding them in favor of the novel objects, depending on the paradigm (delayed matching or nonmatching-to-sample, respectively). Thus, somewhere in the visual system the single presentation of a series of complex stimuli leaves traces against which a subsequent presentation of those same stimuli can be matched. If they do match, i.e. if the original neural traces are reactivated, there is immediate recognition of familiarity. The area in which the neural traces are first established appears to be area TE, since removals here but not elsewhere in the visual system abolish the animal's ability to recognize objects that it has seen once just a few seconds before. Apparently, area TE contains the traces laid down by previous viewing, and these serve as stored representations against which incoming stimuli are constantly being compared. In the process, old traces may either decay or be renewed or even refined, while new traces are added to the store.

Significantly, area TE projects directly to the amygdala and indirectly to the hippocampus via perirhinal and entorhinal cortex. In our previous work we had established that at least part of the neural circuit necessary for storing the traces includes the cortico-limbo-thalamic system, which is actually composed of two largely independent systems arranged in parallel. One of these systems consists of the amygdala, amygdalofugal pathways, and the magnocellular portion of the medial dorsal nucleus (MDmc), and the other consists of the hippocampus, fornix, and anterior nuclear complex of the thalamus (Ant N). The evidence that these two systems operate in parallel comes from our finding that damage to the amygdaloid and hippocampal systems at any stage (i.e. medial temporal lobe, limbo-thalamic pathways, and medial thalamus) causes a severe loss in recognition memory, but only when the two systems are damaged in combination. Damage to just one of the two leads to only mild recognition deficits, suggesting that either system can compensate for loss of the other, at least as far as recognition memory is concerned. Recent results indicate that combined but not separate removal of the orbital frontal and anterior cingulate cortices, which are related anatomically to the amygdaloid and hippocampal pathways, respectively, will also produce a severe recognition deficit. Thus, the ventromedial prefrontal region appears to constitute yet another stage in the limbic memory system.

Recent anatomical evidence has indicated that the bed nucleus of the stria terminalis occupies an anatomical position within the amygdaloid system that is directly comparable to the position occupied by the mamillary bodies within the hippocampal system. That is, just as the hippocampal formation projects to Ant N both directly and indirectly via the mamillary bodies, the amygdala projects to MDmc both directly and indirectly via the bed nucleus of the stria terminalis. This anatomical correspondence suggests that the bed nucleus and the mamillary bodies may serve analogous functions within the two limbic memory circuits. Since previous work has demonstrated that mamillary-body lesions alone produce only a mild recognition memory impairment, we are now testing whether combined damage to the mamillary bodies and bed nucleus will produce a more severe effect.

Our experimental evidence indicating that combined damage to these two limbo-thalamic systems is necessary to produce a disorder in monkeys resembling the syndrome of global amnesia in man is consistent with most of the neuropathological evidence available on amnesic patients, including patients with temporal lobe resections and those with Korsakoff's disease. One piece of clinical evidence does not seem to fit this view, however, and supports instead the view that damage to the hippocampal formation alone is sufficient to produce the syndrome. The evidence comes from amnesic patients with diseases of the posterior cerebral artery, which is known to provide the blood supply of the hippocampus but not the amygdala. From the few clinical studies that included neuropathological reports, however, it appears that although the infarction always includes the posterior part of the hippocampal formation, it may also invade the inferior temporal cortex, stria terminalis, thalamus, or combinations of these, raising the possibility that hippocampal damage alone is not responsible for the memory loss. To examine this issue experimentally, we have initiated a study on the effects of occluding the posterior cerebral artery in the monkey. Our preliminary findings indicate that such occlusion will produce substantial visual recognition loss in some monkeys but not all. Correlation of these findings with the neuropathology may thus resolve the question regarding the locus of damage responsible for the amnesia in patients with posterior cerebral artery occlusions.

The cholinergic system and recognition memory

In the neural model that we have proposed to account for recognition memory, a stimulus leaves a trace in the sensory modality's higher order processing stations whenever that stimulus activates the cortico-limbic pathway described above. This circuit is incomplete, however, because it does not specify the link through which the limbic structures reactivate the sensory cortical areas that activated them. One possible candidate for this missing link is the basal forebrain cholinergic system. Three major lines of evidence support this view. First, cholinergic agonists and antagonists have long been known to influence many forms of memory in many species, and in a number of studies of our own we have found that the same holds true for various forms of memory in the monkey. Second, recent neuropathological studies in patients with Alzheimer's disease, who often show a marked memory loss as one of their earliest symptoms, revealed selected cell loss in both the nucleus basalis of Meynert (nbM), the major source of cholinergic input to the cerebral cortex and amygdala, and the nuclei of the medial septum and diagonal band of Broca (ms/dbB), the major sources of cholinergic input to the hippocampus. This cell loss is accompanied by markedly decreased cortical and limbic levels of choline acetyltransferase and acetylcholinesterase, enzymes involved in the synthesis and metabolism of acetylcholine. Finally, in studies aimed at providing a more complete picture of the anatomical relations between the limbic system and the basal forebrain, we have found that the amygdala and hippocampus project mainly to the same parts of the basal forebrain that innervate them, namely, the nbm and ms/dbB, respectively, with little overlap between them. Interestingly, because the visual system itself does not project directly to the basal forebrain, a relay through the limbic system is essential if the visual system is to activate the cholinergic mechanism.

In experiments performed in collaboration with investigators from The Johns Hopkins Medical School, we produced recognition memory impairments in monkeys by damaging the basal forebrain with a neurotoxin. The monkeys with the most severe memory impairment had nearly complete destruction of the basal forebrain cholinergic nuclei as well as a 60-90% decrease in choline acetyltransferase activity across most of the cortex. However, there were only small decreases of this critical enzyme in area TE, the cortical sector that is so important for visual recognition memory. The results suggest the interesting possibility that the visual recognition impairment resulted from cholinergic denervation not of the cortex but of the limbic system. This possibility would also account for the finding that only combined damage of nbM and ms/dbB yielded impairment, since only such combined damage would result in cholinergic denervation of both the amygdala and hippocampus.

An extremely puzzling aspect of the recognition ability of the animals with basal forebrain lesions is that their performance recovered fully after several months of testing. Because the cortical markers of cholinergic activity did not recover during the same period, the results raise the possibility that other neurotransmitter systems may have compensated for the loss of the cholinergic system. Results from other research on cortical plasticity suggest a compensatory relationship between the activities of norepinephrine and acetylcholine. Although it is a completely open question as to whether norepinephrine is the neurotransmitter involved, loss of neurons in the locus coeruleus, the major source of noradrenergic input to the cortex, has been reported in cases of Alzheimer's disease. In the future, therefore, we plan to investigate the mnemonic effects of depleting the cortex of both acetylcholine and norepinephrine in combination.

In conjunction with these experimental manipulations of the basal forebrain cholinergic system, the goal of which is a better understanding of the nature and neurobiological basis of the cognitive losses associated with Alzheimer's disease, we have begun to examine the cognitive losses associated with normal aging in the monkey. Like the neurotoxic-lesion study, this one also is being performed in collaboration with investigators from The Johns Hopkins Medical School. Our initial results have demonstrated a moderate but systematic decline in the recognition memory of monkeys from early adulthood (3-6 yrs), through middle age (14-17 yrs), to old age (26-30 yrs), as well as an age-related impairment in the type of spatial memory measured by the classical delayed response test. Since there was no correlation between the object recognition and spatial memory deficits in the aged animals, and since the two abilities are known to depend on largely different neural substrates, it is likely that multiple neural systems are vulnerable to the effects of aging and that the vulnerabilities differ from animal to animal. In the future, we plan to test this hypothesis directly by microscopic examination of the tissues in question.

In a related study, we have now mapped the distributions of nicotinic and muscarinic cholinergic receptors in the adult monkey brain. Both types of receptors are found in all cortical areas, but the muscarinic receptors are more widely distributed across the layers of a given field and have a wider variety of laminar labeling patterns across fields than nicotinic receptors. Nicotinic receptors are found predominantly in the deep part of layer III, where incoming thalamic and corticocortical afferents terminate densely, in keeping with the pattern characterizing the forward projections of the sensory processing pathways. Muscarinic receptors, by contrast, are commonly found most densely in the upper and lower layers of the cortex, a pattern that suggests a muscarinic role in the central modulation of sensory processing via the backward projections of those same pathways. The receptor distribution patterns thus appear to fit the notion that the mnemonic effects of the anticholinergic agent scopolamine are exerted through a blockade of the central modulating system.

Molecular mechanisms of neural plasticity

It is likely that release of acetylcholine into the synapses of neurons in the sensory processing pathways initiates a cascade of cellular neurochemical events that leads to the strengthening of the synapses. As a result, many of the neurons whose signals have just represented a sensory stimulus may become linked together in a cell assembly that serves as the stored representation of that stimulus. One cellular process that has become a strong candidate in the search for a molecular mechanism underlying neural plasticity is the phosphorylation of a protein band, F1. This protein band was found by investigators at Northwestern University to increase its rate of phosphate incorporation in the hippocampus both after memory formation and after long term potentiation. In collaboration with these investigators, we have found a protein in the cerebral cortex of the monkey that appears to be homologous to F1 on the basis of molecular weight, isoelectric point, two-dimensional phosphopeptide maps, and phosphorylation by exogenous, purified protein kinase C. To determine whether regional phosphorylation levels of this protein might be related to information storage, we examined protein phosphorylation along the length of the occipitotemporal visual processing pathway. We found an increasing gradient of phosphorylation levels of protein F1, selectively, along the pathway, with levels in rostral inferior temporal and entorhinal cortices approximately ten times those in striate cortex. F1 phosphorylation could thus constitute one mechanism for the cortical storage of visual memories, and further collaborative studies are being planned to investigate this exciting possibility.

A cortico-limbic pathway in somesthesis

The work described above elucidating a cortico-limbo-neuromodulatory system for visual perception and memory has led to the search for analogous systems in other modalities. In fact, analogous anatomical pathways have been tentatively identified in all of the sensory modalities, but because of a recent finding that combined amygdalo-hippocampal lesions produce severe recognition loss not only in vision but also in somesthesis, particular attention has been paid to the pathway in this modality. Also, anatomical studies on the somatosensory system have been supplemented with electrophysiological studies in order to permit functional comparisons with the memory circuit in vision.

Anatomical tracers were injected into the hand representations of physiologically identified somatosensory cortical fields in the postcentral strip and within the lateral sulcus, and the patterns of connections between fields were assessed. Applying principles worked out in the visual system regarding direction of information flow based on analysis of laminar projection patterns, and from our own work with combined lesion and recording studies, we have determined the direction of flow of somatosensory information. Starting from the fields comprising primary somatosensory cortex in the postcentral strip, information is relayed along two pathways, one running dorsally through area 5 and then to area 7, and another coursing ventrally to SII and then onto the insula before reaching the limbic structure of the temporal lobe. This latter pathway is believed to be analogous to the ventrally directed object recognition pathway in the visual system.

In the course of identifying this somatosensory cortico-limbic pathway, we resolved a longstanding difficulty for our serial cortical processing hypothesis, which predicts that the postcentral cortex and SII cortex process information in sequence. It was supposed earlier that these two cortices were both major projection zones of the ventroposterior nucleus, the primary somatosensory relay of the thalamus, implying that the two cortical areas received and processed information in parallel. Two major findings support our view that SII receives its major source of activation from the cortical fields in postcentral cortex as opposed to the thalamus. The first is that following injections of tracers into SII cortex, very few labeled cells are found in the ventroposterior nucleus, demonstrating the lack of a major output from this portion of the thalamus to SII. The other finding is that following removal of the somatic fields comprising postcentral cortex, neurons in SII are no longer responsive to somatic stimulation, indicating the lack of a driving input directly from the thalamus. The two findings together strongly support our suggestion of a sequential corticocortical processing pathway for somethesis. New physiological and behavioral investigations are being directed at the insula, since our anatomical data point to this cortex as constituting the next station in a cortico-limbic pathway for tactile recognition.

Amygdala, hippocampus, and associative memory

According to our neural model, once the trace or representation of a stimulus has been stored in the higher order processing stations of any given modality, that stored trace can enter into association with the stored traces of other stimuli and other events, thereby providing the stimulus with associative meaning. As has been indicated, the amygdala and hippocampus, as well as their separate efferent pathways and separate thalamic targets, make approximately equal contributions to recognition memory, presumably reflecting their roughly equal contribution to the cortical storage of stimulus traces. In the case of associative memory, however, our results indicate that the amygdala and hippocampus make very different contributions.

In one experiment, monkeys were trained preoperatively on a visual recognition task and, separately, on a tactual recognition task, with the same set of objects comprising the stimuli for both modalities. One group of monkeys then received amygdalectomies and the other, hippocampectomies, after which both were retrained on the intramodal memory tasks to a high level of performance. When tested later for their ability to perform the recognition task across modalities, i.e. to choose between two objects visually after one had been presented as a tactile sample, the hippocampectomized monkeys continued to perform at a high level, whereas the performance of the amygdalectomized monkeys fell to chance.

Nearly the opposite results were obtained in a second study that tested the ability of monkeys to remember the spatial location of visual objects. In this task, the animal was required to remember on the test trial where on a three-well tray each of two different objects had been presented on the acquisition trial. In this case, monkeys given amygdalectomy were able to regain the level of performance they had achieved preoperatively, whereas those given hippocampectomy failed to rise above chance.

The results of these two complementary experiments indicate that, although both the amygdala and hippocampus are critical for certain forms of associative memory, their roles are totally different. Many further analyses along the lines of these experiments will of course be necessary before the selective associative memory functions of the amygdala and hippocampus can be identified with confidence. For example, the association of an object with an affective state, such as fear or pleasure, appears to depend much more heavily on the amygdala than on the hippocampus. New support for this view has been obtained in an experiment showing that one-trial object-reward association is impaired more by amygdaloid than by hippocampal lesions, although neither deficit approaches in severity the one produced by the combined removal of these two structures. By contrast, because of the contribution to spatial memory that is made by the hippocampus, the association of objects with spatially directed motor acts could depend more heavily on the hippocampus than on the amygdala. Studies to examine this possibility are being planned.

In one new study that is currently underway, monkeys are being trained on a visual-visual associative memory task. The finding of impairment after amygdaloid but not after hippocampal ablations would imply that the amygdala is critical for stimulus-stimulus associations in general and not just for those that cross modalities. The opposite outcome would suggest, in contrast, that the amygdala is critical for crossmodal associations only and that the hippocampus is responsible for all intramodal associations, with those between object quality and place constituting but one example.

Nonlimbic structures and habit formation

On all of the memory tasks that have been described, the deficits are especially severe when removals of the amygdala and hippocampus are combined. Yet, even the combined limbic lesion does not affect all forms of learning and retention. For example, despite their rapid forgetting in one-trial object recognition, animals with the combined limbic lesions have no difficulty learning object discriminations, at least in the standard situation where trials are repeated 3-4 times per minute. In an attempt to resolve this discrepancy between rapid forgetting and successful learning, we tested whether object discrimination learning would be prevented in animals with limbic lesions if intertrial intervals exceeded the putative memory span. Surprisingly, amnesic animals with the combined amygdalo-hippocampal lesions learned to discriminate a long list of object pairs even though the list was presented only once every 24 hours. The same result was obtained in amnesic animals with combined orbital frontal and cingulate lesions. Thus, although the operated animals have an extremely short memory span, they can retain and accumulate information gained from single discrimination learning trials separated by 24-hour intervals.

Soon after discovering this phenomenon in tests with objects, we found that the same dissociation, i.e. impaired recognition but spared discrimination learning despite 24-hour intertrial intervals, holds for pictorial stimuli as well. This paradoxical success in the presence of severe memory loss implies the existence of a powerful learning and retention mechanism outside the limbic system.

We have since performed additional experiments to characterize further the essential difference in function between the limbic and nonlimbic mechanisms. Our results suggest that the limbic system is critical for high levels of retention of object-reward associations after a single acquisition trial with short lists of objects, or after two or three repetitions with long lists of objects but short intertrial intervals. With greater repetition, however, retention of object-reward associations can be mediated in the absence of the amygdala and hippocampus, and the retention appears to be independent of both list length and delay. To distinguish this form of retention from memory, we have labelled it 'habit formation'. Further investigation of this mechanism of habit formation as well as elucidation of its neural substrate have become important goals of our research.

For example, we have recently begun to explore the projections from the cortex to the neostriatum. So far, we have found that areas TE, TEO, and V4 all project to the tail of the caudate nucleus and to the ventral portion of the putamen. This arrangement contrasts with the pattern of projections from the occipitotemporal pathway to the limbic system, which arise from TE only. The presence of direct projections to the striatum but not to the limbic system from areas V4 and TEO may explain the ability of monkeys with TE lesions to acquire visual habits but not cognitive visual memories. Our aim now is to identify the structures to which the visual portions of the neostriatum project, and ultimately to delineate the entire wiring diagram of the postulated habit system. The structures in the network will form the targets for interventional neurobehavioral studies of the postulated system.

Ontogenetic development of memory and habit formation

Our evidence from studies in adult monkeys suggests that memory formation and habit formation are two qualitatively different retention processes based on separate neural mechanisms. Our recent studies of behavioral development in infant monkeys provide complementary evidence by suggesting that these two systems are developmentally dissociable, in that the nonlimbic habit system appears to mature considerably earlier than the limbic memory system. On the evidence that the limbic memory system is essentially nonfunctional in infants, we have prepared monkeys with neonatal removal of the limbic system (i.e. combined amygdalo-hippocampal removals) in an attempt to see how cognitive, emotional, and social behaviors develop in animals whose amnesia might persist from infancy through adulthood. Animals with neonatal removals of area TE are serving as controls. With regard to habit formation, the results so far indicate that, whereas female infant monkeys can form a set of visual discrimination habits almost as quickly as adults, male infants are significantly retarded. In addition, whereas limbic lesions in both infants and adults leave habit formation intact, neonatal ablation of area TE impairs the learning of female but not of male infants, even though monkeys of both sexes are impaired when the lesions are made in adulthood. These data suggest that ontogenetic development of the habit system is sexually dimorphic, this system maturing earlier in females than in males, presumably because, at this age, area TE or some connected region is more fully developed in females than in males. This sexual dimorphism seems to be dependent on the high testosterone levels found in male infants before and shortly after birth, since a significant correlation appeared between their testosterone levels and learning scores (the more testosterone the poorer the performance), and also because orchiectomy in male infants actually speeds their rate of habit formation.

With regard to cognitive memory formation, the infants with limbic lesions are severely impaired at 10 months of age, whereas those with TE lesions show significant functional sparing (compared to adults with TE lesions). The results thus point to a greater compensatory potential after neonatal cortical than after neonatal limbic removals. The results are consistent with the notion that association areas of the cortex are less mature at birth, and may thus possess greater plasticity, than limbic structures. Direct evidence of neocortical immaturity in the macaque has been provided by our neurobiological studies showing that (a) the distribution of both opiate and muscarinic cholinergic receptors is adult-like at birth in subcortical structures and allocortical areas but is not yet fully developed in neocortical areas, particularly the association cortex and (b) adult levels of metabolic activity in visual association cortex are not reached until about 4 months of age. These behavioral and neurobiological findings suggest that the relatively poor recognition ability in normal neonates is due more to slow maturation of the cortical association areas than to neonatal immaturity of the limbic system.

Because severe recognition impairments follow combined amygdalo-hippocampal removals in infant monkeys just as they do in adults, we investigated whether this severe memory loss from birth onward is accompanied by socio-emotional abnormalities in the developing monkey. At the age of six months, animals with combined amygdalo-hippocampal removals displayed abnormalities such as increased locomotion, decreased manipulation of toys, towels, and cage surfaces and attachments, increased withdrawal from social contacts, and increased finger-sucking. This syndrome was fractionated by the partial limbic lesions, in that only increased locomotion and decreased manipulation were found in the animals with amygdaloid lesions, whereas only increased withdrawal from social contacts was found in the animals with hippocampal lesions. By contrast, the animals with area TE lesions exhibited no significant abnormalities, except for decreased finger-sucking. The results indicate that neonatal removals of amygdala and hippocampus in infant monkeys result in socio-emotional disturbances that resemble those found in autistic children. These abnormalities, together with the recent report of neuropathology in the amygdala, hippocampus, and cerebellum of the brain of an autistic man, provide evidence that early dysfunction of the limbic system may be one cause of infantile autism.

Our plan is to follow the psychological development of our neonatally operated animals into adulthood. The results of the study will help to evaluate two provocative proposals from the clinical literature, namely, (a) that early dysfunction of the limbo-thalamic memory system could be one cause of childhood autism, a syndrome characterized by dramatic social and emotional disturbances not seen in adults with the same neuropathology, and (b) that the reason a pure case of amnesia like the one seen in adults has never been reported in a child is that the clinical picture of an amnesic child, being overlaid with autism, is entirely different from the clinical picture of an amnesic adult.

Summary

Through combined use of behavioral and neurobiological methods, we are beginning to discover some of the general principles along which the primate forebrain is organized to serve memory and other cognitive processes. (1) Each primary projection area in the cortex seems to be the source of two multisynaptic corticocortical pathways. Both pathways are composed of several cortical areas that are arranged hierarchically, one pathway being directed dorsally to the frontal motor system, the other ventrally to the temporal limbic system. Before reaching the motor system, the dorsal pathways from the several modalities converge in polysensory areas, which are critical for sensory attention, spatial perception, and motor guidance. The ventral pathways, by contrast, remain modality specific throughout their course and are important instead for stimulus recognition and stimulus meaning, and

ultimately for triggering the motor response. (2) Stimulus recognition depends not only on stimulus processing along the ventral cortical pathway but also on storage of a central representation of that stimulus in the ventral pathway's last station, located in the anterior temporo-insular region. This region projects directly to the amygdala and indirectly to the hippocampus, and these two limbic structures project in turn to the medial thalamus, with further connections to ventromedial prefrontal cortex. All three of these regions (limbic, thalamic, and prefrontal) project to the basal forebrain cholinergic system as well as other neuromodulatory systems, which innervate the entire cortical mantle. It is hypothesized that storage of the central representation of a stimulus occurs only if this cortico-limbo-neuromodulatory circuit is activated. Damage to this pathway results in recognition failure, a core symptom of amnesia. (3) Once the central representation of a stimulus has been stored, it can enter into association with the stored central representations of other stimuli and other events, thereby providing the stimulus with meaning. Thus, stimulus-stimulus associations appear to depend on cortico-amygdalo-cortical interactions, while stimulus-affect associations probably depend largely on cortico-amygdalo-hypothalamic interactions. By contrast, stimulus-place associations seem to depend on cortico-hippocampal interaction; in this case the hippocampus may act as the site of converging information from both the ventral 'stimulus recognition' pathway and the dorsal 'spatial perception' pathway, the latter involving projections from the polysensory areas through the cingulate gyrus to the hippocampus. Finally, stimulus-act associations could depend on interaction, via the limbic system, between the ventral and dorsal pathways within the lateral prefrontal cortex, which interacts in turn with the motor system. (4) Destruction of the limbic portion of the memory system does not affect all forms of learning and retention, however. At least one form, which has been labeled habit formation, remains nearly intact, presumably reflecting the operation of a powerful cortico-nonlimbic mechanism for retention of stimulus-response connections. Judged by the evidence from our behavioral and developmental neurobiological studies, this nonlimbic habit system appears to mature considerably earlier than the limbic memory system. We have postulated that the nonlimbic habit system depends largely on interaction between the cerebral cortex and the basal ganglia.

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Annual Report of the Laboratory of Developmental Psychology

National Institute of Mental Health

October 1, 1985 through September 30, 1986

Marian Radke-Yarrow, Chief

The research of the Laboratory of Developmental Psychology is focused primarily on developmental psychopathology. A first unifying theme in the research program is development, per se. Behaviors and relationships that are regarded as psychopathological or adaptive are investigated with reference to the developmental stage of the child, and with longitudinal methods. The mechanisms underlying the patterns of developmental changes in child behavior are of particular interest.

A second broad question concerns interrelations of environmental and constitutional or genetic factors in determining behavioral development. Both the environments and the expression of inherited propensities change over the life of the child. To begin to adequately address this domain of inquiry, therefore, also requires that these issues be considered within a developmental framework.

A third emphasis is on methodology. We are much concerned with measurement of environment in relation to child development. Improved measures are necessary if we are to be able to say which environmental variables have an impact on which domains of child behavior. Another methodological issue involves the development of diagnostic procedures for use with very young children.

These questions are addressed in three program projects: (1) the development of children at risk of pathology by virtue of their parents' depression, (2) problem behaviors in young children and their predictive value for later development, and (3) problems of adolescents.

The Development of Children of Depressed and Normal Parents

The major goals of the study of children of depressed and normal parents are (1) to identify salient aspects of parental behavior that distinguish depressed and normal parents, (2) to examine relations between the behavior of depressed parents and the children's development of adaptive and maladaptive behavior patterns (including depression), and (3) to be able to describe prospectively the developmental course of depression.

The families in this study (N=123) are those in which the parents have the diagnosis of unipolar, minor, or bipolar depression, or have no history of psychiatric disorder. Each family has two children one of whom at time of entrance into the study, is 2 to 3 years and one who is 5 to 8 years of age. The families are to be followed until the children reach adolescence. Multiple evaluations are made of the parents and children. (For design and methodology, see Z01 MH 02144.) Findings, to date, are based on the first period of assessment and on the 80 families who have thus far been seen in the first follow-up.

Psychiatric assessments provide one important kind of evaluation of the children (Drs. Leon Cytryn, Donald McKnew, Tracy Sherman, and Marian Radke-Yarrow). The 2- to 3-year-olds were assessed using two structured behavioral observation systems: in a psychiatric play interview in the absence of mother, and in interaction with mother. Each child was assigned a rating of degree of risk for the later development of psychopathology based on his/her behavior in each of the two assessment settings. The risk assessment is based on a standard method of assessment, which uses behavior ratings and clinical judgments.

The 10 children who received high risk ratings in both evaluation settings have been subjectd to case-by-case analysis. Two of these children are boys from families in which neither parent had a history of psychiatric illness. Of the remaining children (5 boys and 3 girls), five are from families in which both parents have affective disorder, and three from families with one ill parent. Two major constellations of behavioral symptoms are in these high-risk toddlers. One consists of an isolated or distant type of relationship to mother, dysphoria, and anhedonia. The other consists of an angry type of relationship with mother, and predominantly angry mood. The children in both groups demonstrate dysregulation of emotions under stress.

A standard psychiatric interview (Child Assesment Schedule) and diagnoses according to the DSM-III criteria are used for children five years of age and older. Psychiatric status as a function of parental psychopathology was examined. More of the children whose parent(s) have an affective disorder than whose parents are normal receive DSM-III diagnoses. The difference reaches significance for the younger children at the second time of assessment. This trend did not reach significance for the older siblings at either the first or second round of assessments. Also, more children whose parents have major affective disorder received diagnoses at both times of assessment than did children whose parents have no history of psychiatric disorder.

Longitudinal assessment of the children of affectively ill and healthy parents will enable us to identify ages at which the appearance of affective disturbance is common, but not predictive of continued psychopathology, and hopefully, points in development when the appearance of affective disturbance is predictive of continued psychopathology.

In another evaluation of the children, early childhood inhibition is being investigated as an index of risk for pathological development. It is being studied in children of families with and without parental depression (Drs. Frances Bridges-Cline and Grazyna Kochanska). Patterns of child behavior in the face of the unfamiliar persons and/or places are observed in semi-naturalistic but standard settings, which represent varied contexts of unfamiliarity. Behaviors expressing inhibited exploratory activity and social withdrawal are noted when the children are 2 to 3 years of age and again when they are 5 to 6 years of age. Comparisons of behavioral inhibition across maternal diagnostic groups of normal, major depression, and bipolar indicate that the young children of the major depressive mothers in our sample typically exhibit the most inhibited forms of behavior. Children of the bipolar mothers typically exhibit the most active and independent forms of response when the situation is that of an unfamiliar environment. However, in the situation of an unfamiliar person, the children of the bipolar mothers, as a group, exhibit very divergent forms of response, scoring at both the inhibited and uninhibited polar extremes of the response scales. Longitudinal data are being analyzed to examine the direct and indirect associations of these early response characteristics of inhibition with later manifestations of disordered or healthy behavior at five to six years.

The behavioral environment provided by the depressed and normal parents has been evaluated on multiple dimensions that are assumed to influence the development of affect regulation, social relatedness, and mastery and coping skills in the child; and that are predicted to suffer impairment relating to the parent's affective illness. In these investigations the objective is not simply to determine differences in the environment but to examine the child behaviors that are most likely to be influenced by a disturbed environment and, thereby, to begin to identify processes involved in the development of maladaptive behaviors. Four major components of parenting that have been investigated are (a) the attachment relationship, (b) the control and regulation of child behavior, (c) communication between parent and child, and (d) the affective aspects of interaction.

(a) It was found that the mother-child attachment relationship at two years is more frequently disturbed (insecure) in families in which the mother suffers from bipolar depression or major unipolar depression than in families in which the mother is free of psychiatric problems. How these secure and insecure attachment patterns will continue to evolve over time in the ill and well groups is being analyzed (Drs. E. Mark Cummings and Marian Radke-Yarrow).

(b) Along with the greater risk of disturbance in the basic mother-child relationship, the offspring of depressed parents, as a group, are subjected to parents' impaired control practices. Mothers' discipline and control behavior and their children's responses to maternal control attempts were studied by Drs. Grazyna Kochanska, Leon Kuczynski, and Marian Radke-Yarrow. Impaired parental skills in managing children's behavior were found more often among depressed than among normal mothers. Analyses revealed that the more severely ill the mother the more often she immediately aborts her attempt to control or direct her child's behavior when faced with her child's resistance, the more frequently she forcibly overcomes the child's resistance, and the more likely she is to create unresolvable situations for the child; the less likely she is to accept child's negotiation attempts. Related deficits were found in the child's behavior. Children of depressed mothers are more noncompliant and less able to negotiate with their mother in order to effect some change in her demand of them. Children of normal mothers, but not of depressed mothers, become more cooperative in the period from 2 to 3 1/2 years. In general, in the area of maternal regulation of child behavior, dysfunctional parent-child relationships are significantly more frequent in the depressed families.

Paternal contributions in families in which there is maternal depression do not appear to provide a general corrective. In about half of the families the father, too, has a diagnosis of depression. Well fathers with well spouses describe fathering as involving regularly occurring contact with their children that is most often father-initiated, occurs at home, and involves intimate activities, e.g., reading stories or carrying on a conversation. On the other hand, well fathers with ill spouses describe events that are "special" non-routine, are child-initiated, occur away from home, and involve physical play or outings (Dr. Elbert Wilson).

(c) Verbal communication between mothers and children can be a sensitive indicator of their relationship and also of what the parent is transmitting to the child. Two projects were undertaken; one is an investigation of the patterns of speech between mother and child without reference to content (Drs. Zvia Breznitz and Tracy Sherman); the other is an analysis of content of mother's and child's speech (Dr. Marian Radke-Yarrow, Barbara Belmont, and Anne Mayfield). Both projects have identified features distinguishing normal and depressed parents and their children. Given the general motor retardation, the reduced energy level and social withdrawal of depressed individuals, it was predicted that the speech patterns of depressed mothers would differ from the speech patterns of well mothers. Depressed mothers were found to vocalize less often than healthy mothers. Also, the children of the depressed mothers compared with children of normal mothers speak much less to their mother in a situation intended to be a relaxed social setting eating lunch together. However, in a situation intended to place mild stress on the dyad (i.e., awaiting a doctor's visit), the depressed women change their speech pattern to one of increased production. Similarly, their children's speech increases in amount to more closely approximate that of normal children. This change does not occur in the speech of the children of healthy mothers. The change is interpreted as depressed dyads' more dysregulated reactions under stress. The overall pattern of behavior suggests that depressed and normal mothers interact verbally differently with their children. It is hypothesized that the reduced frequency of vocalizations by depressed mothers, except under stress, may reduce the reinforcing and socializing capacity of speech for their children. The differences in the behavior between the two groups of children indicate that these influences are already apparent and affecting the behavior of the three-year-old offspring.

The second study focuses on speech content in the natural discourse between mothers and children. The early development of children's self-conceptions is believed to be heavily determined within the family. The mother's comments to and about her child are one plausible contributor to the child's self-knowledge and self-regard. For children of depressed parents, self-evaluations are of special significance because self-attributions of hopelessness, helplessness, and unworthiness are common in depressed adults. There is the possibility that depressed parents convey such negative messages to and about their children, beginning when the children are very young.

The findings support expectations of group differences. Depressed mothers make significantly more negative comments to their children than do normal mothers, and make comments with ambiguous and affectively mixed messages. Normal mothers more than depressed mothers talk to the child in terms that emphasize the child's identity and individuality.

Mother's attributions to the child about him/herself and the child's self-references are related in a number of ways. The frequency of mothers' attributions to their children is significantly positively correlated with the frequency of children's self-references, within both the normal and depressed groups. In depressed mother-child dyads, the frequency of negative attributions by the mother is positively correlated with the frequency of negative self-references by the child. In the group of normal mothers-child dyads, secure attachment patterns are positively related to frequency of positive self-references by the children. In the depressed group, insecurity of attachment is associated with reduced numbers of comments to the child by the mother.

The differences in the groups indicate that differences in basic communication patterns are already manifest in the relationship of the three-year-old and his/her mother -- that both developmental research and theory would predict will have pernicious effect on the child's continued social and emotional development.

(d) Affective dimensions of normal and depressed mothers' and their children's behavior are investigated. The team of investigators for the affective analyses includes Drs. Marian Radke-Yarrow, Leon Kuczynski, Grazyna Kochanska, Elbert Wilson, and Editha Nottelmann; and Barbara Belmont, Anna Polissar, and Judy Stilwell. It was hypothesized that children's experiences in the family environment provided by depressed parents would diverge significantly from that provided by normal families with respect to moods and emotions. These expectations are confirmed in many ways. Based on continuous coding of the affect of mother and children over 9 hours of observation in planfully varied situations, the following significant group differences were found: There were ratings of negative mood or specific negative emotions, (sad, fearful, angry, or anxious) in significantly more minutes for depressed mothers and their 2- to 3-year-olds and 5-to-7-year-olds than for normal mother-child dyads and triads. The quality rather than the frequency of physical affection expressed by mothers differs in the normal and depressed groups. For a subgroup of severely depressed mothers there is prolonged, sometimes sensual affection mixed with tension and sadness. For a subgroup of manic-depressive mothers, physical affection is attempted, which was at times inappropriate to the child's needs. Moreover, physical affection becomes "severe" and aversive. There is very high co-occurrence (matches by minute) of affective expression by mother and young child, particularly for depressed mothers and their daughters.

In summary, we have found differences in parenting behaviors in depressed and well mothers and fathers that appear to be interfering with the child's achieving a secure base in the parent, a positive conception of self, with the child developing positive social relationships, with the child's learning control and compliance, and with the child's developing capacities to experience positive affect and to regulate emotions.

This program of analyses of the environments provided by depressed and normal parents will continue. There will be longitudinal analyses of data from the follow-up assessments. Additionally, types of data have been collected, will be added to the currently available observational data. Important among these are the genetic histories and life-event histories of the families.

A project that extends one part of this program is an examination of interactions of depressed mothers and their infants. It has been initiated by Dr. Kathryn Suter and Judy Stilwell. In a laboratory design, affective regulation, attachment behavior, and patterns of mother-infant interaction in infants of four groups of parents are studied: bipolar mothers and well fathers, bipolar fathers and well mothers, chronically depressed mothers and well fathers, and both parents without psychiatric illness. Families are seen on two occasions five months apart, beginning either when the infants are aged 3 months, 8 months, or 13 months.

Problem Behaviors of Young Children

Drs. Carolyn Zahn-Waxler, E. Mark Cummings, and Ronald Iannotti are investigating internalizing and externalizing problems of young children, and examining the prediction from behaviors at age two to behavior problems when the child reaches school-age. Forty-eight children of normal and depressed mothers were seen at two years of age and again at six years in laboratory sessions presenting a range of challenging conditions. Children's social and emotional interchanges with mother, with familiar playmates and with an adult stranger were evaluated and also the child's ability to sustain social play, and cope with problems and frustrations. Mothers completed the Achenbach Child Behavior Check List at each time of assessment. When these children were two years of age, parental depression was related both to children's problems of overcontrol and undercontrol. Analyses at six years, based on the Child Behavior Check List, indicate stability over time in these patterns primarily for children with a depressed caregiver. Stability of the patterns is more characteristic for boys than girls. Unfocused aggression at age two predicts externalizing problems and, to a lesser degree, internalizing problems at age six. Compliance and passivity with peers, and excessive dependency on the mother at age two predict later aggression. To a lesser degree, these internalizing symptoms at two years also predict internalizing problems at six years. Good social skills with peers at age two were associated with having few problem behaviors at age six. The investigation of stabilities and instabilities over this span of years is now being investigated in the data generated by the laboratory observations of the children.

Problems of Adolescents

Interrelations of cognitive, affective, and interpersonal competencies and dysfunctions and endocrine and physical growth changes are investigated, cross-sectionally and longitudinally (Drs. Editha Nottelmann and Elizabeth Susman, and Gale Germain and Lorah Dorn, and collaborators in NICHD, Drs. George Chrousos, Gordon Cutler, D. Lynn Loriaux).

The participants are 9 to 14 year old boys and girls and their parents. They are seen three times, six months apart. The markers of pubertal development include serum hormone levels (gonadotropins, sex steroids, and adrenal androgens), pubertal stage (Tanner criteria), and height and weight. Psychological assessments of the adolescent include measures of behavior problems, affective states, self-perceptions, interpersonal functioning, parent-child interactions in standard laboratory situations, and standard assessments of cognitive functioning. Relations between hormone levels and (a) anxiety and depressive symptoms, (b) parent-child interactions, and (c) cognitive functioning have been examined.

Cross-sectional findings at Time 1 on interrelations among biological measures and relations between biological and psychological measures were described in last year's report. Findings on hormone levels generally were replicated cross-sectionally at Time 2 and Time 3. Additional endocrine measures, not included in last year's report, are cortisol (F) and cortisol-binding globulin (CBG), for which large individual differences were found but no sex or pubertal stage differences. Biological changes across Time 1-3 were examined to determine the rate and trajectory of biological change during the one-year period of the study. This information is the first step in the process of examining biological change influences on psychological functioning. Ongoing analyses of predictive relations between Time 1 biological measures and Time 3 psychological measures indicate that higher levels of androstenedione and lower dehydroepiandrosterone sulphate levels at Time 1 continue to be associated with behavior problems at Time 3. Time of maturation as well as rate of biological change (in physical maturity for boys and hormone levels for girls) also predicted self-reported psychological adjustment at Time 3.

Hormone levels of boys, primarily of gonadal steroids and of adrenal androgens, were predictive of anxious and depressive affect one year later. Hormone levels, per se, were not predictive of number of symptoms one year later in girls. A more rapid rate of change (across one year) in level of adrenal androgens in boys and of gonadotropins in girls was accompanied by a higher incidence of anxiety and depressive symptoms at the year's end. Cross-sectional analyses of relations between hormone levels and the adolescent's use of anger and power observed in parent-adolescent interactions indicated that boys with higher levels of androstenedione were higher on explosive behavior. Girls with higher levels of androstenedione were higher on dominance, defiance, and expression of anger toward both mother and father.

Cross-sectional and longitudinal analyses will continue through the coming year.

Activities of Laboratory Investigators

A number of investigators in the Laboratory have, for the fourth year, received support from the John D. and Catherine T. MacArthur Foundation for research on developmental processes.

Investigators in the Laboratory have been very active in the scientific community, in the United States and Internationally, giving invited presentations of Laboratory work at conferences and professional meetings.

Annual Report of the
Laboratory of Psychology and Psychopathology
National Institute of Mental Health

October 1, 1985 to September 30, 1986

Allan F. Mirsky, Ph.D., Chief

This is the sixth full year report of the program of the Laboratory of Psychology and Psychopathology (LPP). A number of administrative changes have been made which affect the LPP: Dr. Coppola has been reassigned to the Adult Psychiatry Branch at St. Elizabeth's Hospital, although he continues some of his activities in the Clinical Center. Dr. Nakamura has assumed an extramural position with the Division of Basic Research; he remains a Guest Researcher in this laboratory. As of October 1, 1986, Dr. Weingartner's remaining NIMH IRP activities will be conducted under the aegis of the Laboratory of Clinical Sciences. Dr. Silberman transferred in June 1986 to the Hahnemann Medical College in Philadelphia. We plan to add a developmental psychophysiologist, Dr. Bruno Anthony, to our staff this year. He will participate in studies of children at risk for psychopathological disorders being conducted in conjunction with the School of Public Health at Johns Hopkins University in Baltimore. In addition, a Guest Researcher from Johns Hopkins, Dr. Frances Gabbay, is now pursuing studies of heredity-environment interaction in our laboratory.

We have occupied our permanent laboratory space in the ACRF since May of 1983; our second major human ERP laboratory is now functional and we will begin testing children from various clinical populations in September of this year. Several hundred patient sessions have been run by LPP investigators during the reporting period, and there have been a number of exciting findings.

A brief outline of studies appears below.

A. Clinical Studies of Attention and Brain Function

1. Human Clinical Studies of Attention Disorder
2. Attention Disorders as Assessed by Event-related Brain Potentials
3. Neuropsychological Evaluation of Psychiatric and Neurological Patients
4. Topographic Analysis of Brain Activity

B. Animal models of attention disorders

1. Models in the Monkey of Generalized Seizures of the Absence Type
2. Brain Lesion and State Change Effects on Visual Attention

C. Psychobiology of Cognitive Processes

D. Autonomic Nervous System Activity in Attention and Psychopathology

1. Psychophysiological Responsivity and Behavior in Schizophrenia
2. Psychophysiological Concomitants of Minimal Brain Dysfunction in Children
3. Personality Factors and Psychophysiological Responses to Changing Stimulus Input

E. Interaction of Nature and Nurture in Schizophrenia

1. Studies of Heredity and Environment in Schizophrenia
2. Studies on Etiological Factors in Schizophrenia

F. Cognitive and Perceptual Changes in Affective Illness

A. Clinical Studies of Attention and Brain Function

1. Human Clinical Studies of Attention Disorder

Many of the members of the LPP are involved in a joint project in which a wide variety of attentional, cognitive, autonomic, and electroencephalographically-derived tests are applied to the same populations of experimental and control subjects. These will include epileptic persons, schizophrenic subjects, brain-lesioned subjects, dementing subjects, and controls. The aim is to develop a profile of functioning for the several groups that will highlight the similarities and differences among them and will lead to a better neuropsychological characterization of their impairment.

The major elements within this program include: the work of the Cognitive Psychophysiology Unit (Dr. Connie C. Duncan, head); the Neuropsychological Test Battery by Drs. Goldberg and Jones (consultants) and Dr. Duncan. The bulk of the neuropsychological examinations are conducted or supervised by Dr. Emile Brouwers. Dr. Duncan's Unit is applying techniques of cognitive psychophysiology to elicit and evaluate the several event related potential components (auditory and visual) which have been shown to relate to attention, uncertainty and surprise (P300, N140, etc.). Similar experimental paradigms (e.g., the "odd-ball" method) have been applied to various groups of subjects with attentional disorders (eating disorders, schizophrenia, dyslexia, etc.) so as to be able to compare and contrast ERP component amplitudes, latencies and distributions. It is anticipated that these studies will help to illuminate (in conjunction with other methods) the nature of the similarities and differences among various attentional disorders.

2. Attention Disorders as Assessed by Event-Related Brain Potentials

Major progress has been made this year in testing various groups of disordered patients with ERP techniques. Significant new findings, in particular, have emerged in characterizing and contrasting the cognitive/attentive disturbances in patients with various types of eating disorders, adult dyslexia, and schizophrenia. Patients with normal weight bulimia can be distinguished from those with anorexia of the restrictor type. Dyslexics can be subdivided into two groups (based on ERP findings) depending on whether or not they were hyperactive as children. Further, schizophrenics show major ERP differences as a function of successful drug therapy. Moreover, some important findings have emerged in a study of seasonal affective disorders, suggesting that patients with this disorder process visual information more effectively if they respond to phototherapy. More details are found in the project description of Z01 MH 00509-03 LPP.

3. Neuropsychological Evaluation of Psychiatric and Neurological Patients

The Neuropsychological Test Battery was developed by various LPP staff, including Dr. Connie Duncan, Ms. Kathleen Squillace and outside consultants Dr. Elkhonon Goldberg of Einstein Medical School, and Dr. Barbara Jones. Our battery of neuropsychological tests provides a complete assessment of executive, mnemonic, linguistic and attentive capacities of the human brain. The test battery has been administered in whole or in part to about 235 persons so far, and the information it provides will form the neurobehavioral data base which will be used to interrelate, evaluate and integrate the various electrographic, biochemical and other physiological measures applied to various groups of patients with psychiatric and neurological disorders. Additional details can be found in the project description of Z01 MH 00508-03 LPP.

4. Topographic Analysis of Brain Activity

Dr. Coppola's imaging laboratory became operational in the fall of 1982. Continuing from the work begun with Dr. Buchsbaum a 32 lead continuous EEG mapping system was implemented. The laboratory is now regularly recording 32 lead studies from a variety of patient groups. It appears that in some situations the additional imaging resolution gained from 32 leads vs 16 on one hemisphere can be quite useful. In other cases the 32 lead capability is used to record from both hemispheres simultaneously, allowing bilateral comparisons. Aside from evaluation of this new technique, studies are in progress examining patients with depression (under IV procaine HCL challenge); examining cases with presumed Alzheimer's disease; developing EEG imaging in patients with epilepsy; evaluating the effects of various psychotropic drugs on the EEG; comparing groups with differing major foci of alpha EEG activity.

B. Animal Models of Attention Disorders

1. Models in the Monkey of Generalized Seizures of the Absence Type

Over the course of the last 15 years, staff now in the LPP have been interested in the development of techniques for simulating the generalized seizure patterns, as well as the behavioral accompaniments, of absence epilepsy. We have explored the use of electrical, chemical, and metabolic methods of inducing discharges. Most recently, we have done some experiments with compounds related to the metabolism of GABA (i.e., GBL, GHB). The work has suggested that these particular compounds produce effects which are more like sleep than like absence epilepsy. Nevertheless, such models permit the testing of various hypotheses about neurochemical events in absence epilepsy, and provide an experimental tool for study of single unit activity (as an example) under conditions of seizure.

These studies have been conducted under the direction of Dr. Michael Myslobodsky and with the participation of Drs. Richard Nakamura and Richard Coppola.

2. Brain Lesion and State Change Effects on Visual Attention

There is collaboration with LN and LCM in some parts of this work. One of the goals has been to specify the role of non-visual regions of the primate cerebrum in visual attention, and this work has used several techniques including lesions, stimulation, and electrographic recording of brain potentials under various lesion and non-lesion conditions. Other studies have been concerned with the recording and analysis of visually-evoked potentials under a variety of perceptual conditions, and with drug or sleep state effects on the mechanisms of visual attention.

One of the behavioral measures used in this study is the Generalized Attention Test (GAT) which is being developed at the NIMH by LPP staff. It represents an extension and modification of the Continuous Performance Test (CPT) of attention which was originally refined and perfected at the NIMH during the period 1954-1961. The CPT has been used extensively by various groups of investigators, particularly in the study of epilepsy, schizophrenia, and metabolic illnesses. The GAT extends the concept of the CPT to involve a variety of cognitive dimensions (such as intra and inter-dimensional shifts), as well as parametric control of other perceptual variables. The GAT is designed for use with both humans and monkeys so that direct comparisons can be made of the results in each species. Preliminary results on monkeys indicates that they can be successfully trained to perform on this task and some of the data from the first monkey subjects are extremely promising. They indicate some unexpected allocations of function among sensory, motor, and "association" areas of the monkey cortex.

C. Psychobiology of Cognitive Processes

The aim of this research, conducted under the direction of Dr. Herbert Weingartner, has been to relate psychological and biological determinants of various components of cognition that are involved in the acquisition, processing, retention, consolidation, and retrieval of information or experience.

Experiments have been designed to characterize and contrast cognitive failures in patients with disturbances of mood, children with various forms of impairment in learning, and populations of neuropsychiatric patients including those with Huntington's disease, Korsakoff's psychosis, and Alzheimer's disease. How are specific forms of central nervous system changes related to discrete alterations in cognitive processes? Components of information processing in unimpaired individuals are also studied. A third type of study of both unimpaired individuals and patient groups uses various types of drug manipulation to alter specific aspects of cognitive processes. How do drug treatments that alter the activity of discrete aggregates of neurons relate to qualitatively different changes in cognitive processes? This program of studies has been described in previous annual reports, and will be reported henceforth under the aegis of the Laboratory of Clinical Sciences.

D. Autonomic Nervous System Activity in Attention and Psychopathology

This work is being carried out by Dr. Theodore Zahn. During the year, Dr. Zahn accepted an appointment to the Editorial Board of the *Journal of Abnormal Psychology*.

The central focus of this research is the role of attentional processes and autonomic nervous system (ANS) functioning in psychopathology, especially schizophrenia. Studies are directed toward several basic issues: (1) the nature of the attention and ANS dysfunction, (2) the diagnostic specificity of the dysfunction, (3) state vs. trait issues, (4) the neurobiological basis of attention and ANS functioning.

We are continuing to collect data on our current schizophrenia protocol which is designed to study ANS activity during rest and task performance, compare ANS responses to stimuli differing in signal value with respect to frequency, amplitude, and habituation, and determine the nature of attention deficits in schizophrenia. Efforts are being made to test patients on standard neuroleptic medication as well as drug-free patients because most non-NIMH investigators have only medicated patients available to them. This will provide information as to the extent to which their results are influenced by medication. Recruitment of subjects has been slow due to the slow turnover of Clinical Center patients, and the small number of patients available from other sources. We need to increase our sample of controls as well.

A study of young men who were autistic children has been completed and written up. Age matched acute schizophrenic and normal men were controls. There was some, but rather unimpressive, evidence of high ANS arousal in the autistics, manifested in elevated electrodermal activity (EDA) and low skin temperature. However, the autistics had more significant high respiration rates and high heart rate variability. They were very unresponsive autonomically to performance of a reaction time task and showed a gross attention deficit. In these respects they differed from controls in the same direction as the schizophrenics, but more extremely so. The results are consistent with a hypothesis of brainstem dysfunction in autism but inconsistent with the catecholamine and input dysregulation hypotheses that have been proposed.

We are also slowly building up our sample of patients with affective disorder and may be able to do a formal comparison with schizophrenia in the coming year. We may also have a sufficient number of patients with panic disorder.

During the year, we completed a challenge study of yohimbine, an alpha-2 receptor antagonist, on 12 normal controls and 15 patients with panic disorder, about half of them on alprazolam therapy, in collaboration with Dr. Albus, a Visiting Scientist with NSB, and Dr. Uhde of BPB. As expected, yohimbine had its major effects on cardiovascular activity. It raised heart rate in patients under resting conditions as well as when they were stressed by mental arithmetic (MA) and sustained attention tasks. Controls had only a larger heart rate response to the MA task when on yohimbine but no general increase. However, both groups showed a small increase in serum cortisol and a robust increase on a measure of heart rate variability after yohimbine. The latter finding could be due to an increase in respiratory tidal volume which has been proposed as a progenitor of panic attacks by some investigators. However, although ratings of panicky feelings and anxiety increased in the patients (but not in controls), no patient had a panic attack following the yohimbine. Alprazolam treatment had surprisingly little effect on the data. Further analysis of this data set is in progress. We are eagerly awaiting catecholamine values.

In a study with Drs. Flament and Rapoport of the CHP, we are giving a caffeine challenge to children with anxiety disorders and matched controls. Preliminary results show that electrodermal activity is not increased in the anxiety group by caffeine as it is in the controls, and that there is no difference in side effects. These results are surprising because of the general belief that patients with anxiety disorders are more sensitive to caffeine than controls. This appears not to be the case in children at least. Tests of a few more subjects in this project are underway.

Another major ongoing project in Dr. Zahn's laboratory is a study of the psychophysiological and neuroendocrine effects of a "learned helplessness" procedure which is designed to produce a temporary mood change similar to depression in normal subjects. This is accomplished by presenting subjects with an insoluble task of learning how to terminate bursts of loud noise which are presented periodically. This condition ("nonescape") is compared with one in which the same amount of noise is presented but each burst can be terminated by a learned response. One of the objectives of this study is to see if valid results can be obtained by using each subject as his/her own control, thereby making the procedure more sensitive and useful for small clinical samples. Results to date on normal volunteers show that the two conditions are having their expected subjective effects--subjects rate themselves higher on such items as tension, helplessness, and depression after the nonescape condition. This is accompanied by evidence of higher electrodermal activity, higher plasma ACTH, and a trend for higher cortisol after the nonescape compared to the escape condition. Testing is continuing on controls and on patients with schizophrenia and several varieties of affective disorder. In addition to the obvious implications of this study for understanding the neurobiology of affective disorders, we are interested in comparing the effects of aversive stimuli and the associated learned escape response on the physiological activity of the patients--especially schizophrenics--since if we find a deficit in ANS responding in this situation, a motivational explanation of low schizophrenic reactivity to nonaversive signal stimuli will be less plausible.

This study, as well as the two challenge studies described above, in addition to their main objectives, are also relevant to our goal to better understand the neurobiological mechanisms underlying our ANS measures. We have completed data collection on a collaborative study with Dr. Zohar in LCS directly related to this goal in which subjects with unusually high levels of plasma norepinephrine were compared to those with normal to low levels on our standard ANS paradigm. These data are being analyzed. In addition, we are planning to test patients with focal head injuries being recruited by LPP on the ANS-attention battery we use with schizophrenics. This should help us determine if the deficits we see in schizophrenics could be due to localized brain dysfunction.

Several studies of normal subjects who are either at risk for psychopathology or have traits similar to those seen in some patients are being done or planned. Offspring of patients with bipolar affective disorder are being tested with both ANS and attention measures in collaboration with Dr. Nurnberger of CNG. The purpose is to determine if some of the characteristics reported in the literature for affective disorder, such as low skin conductance and attention impairments, are present in a disproportionate number of the offspring and could possibly qualify as genetic markers. With Dr. Rapoport's group, we are doing a 3-5 year follow-up of obsessive-compulsive children. Since some, but not all, of the subjects are now nonsymptomatic, comparison with their previous tests may provide valuable information about the ANS characteristics that are trait vs. state related in this disorder.

Dr. Robinson, a Guest Researcher in LPP, is continuing his studies on personality and psychophysiology. Recently he has analyzed his data in terms of a published scale of "schizotypal personality" and has found that normal volunteers with high scores on this scale tend to be low on several measures of ANS arousal. These results bear a conceptual similarity to some of his previous results with a well-known measure of "psychotocism", suggesting some generality to these findings.

Finally, we are planning to test the children whom LPP is attempting to recruit from the Hopkins preventive intervention project who have been screened for traits that may be predictive of future behavior problems. On the basis of previous research from our laboratory and elsewhere, we are optimistic that such traits as fearfulness, aggressiveness, and impulsivity may each be distinguishable from well-adjusted by our ANS and/or behavioral measures.

E. Interaction of Nature and Nurture in Schizophrenia

1. Studies of Heredity and Environment in Schizophrenia

The project is composed of the following studies:

a. An intensive multidisciplinary study of a family with monozygous quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome.

The first study of this family was completed and published in book form in 1963. We have continued our contacts with this family to follow the clinical course of these women and to see how the course is related to earlier and current life experiences. A second intensive multidisciplinary study of these women was completed in June of 1981, and has been summarized in previous annual reports. At present, theoretical pieces describing nature-nurture issues in the Genain quadruplets are in press or in preparation.

b. Studies of adoptees with schizophrenic parents and their biological and adoptive families.

This represents a completion of work done with a cohort of adoptees and controls that were obtained in Denmark in the 1960's. Although a considerable portion of this work has been published, much of the psychological information was not analyzed. A reanalysis of the original case material has appeared in the American Journal of Psychiatry. Another paper comparing disordered and non-disordered subjects in terms of reported stresses during development is in preparation at this time.

c. A study of children of schizophrenic and control parents reared in town or kibbutz in Israel.

This study, begun in 1965, has involved a multidisciplinary (psychological, psychiatric, neurological) examination of a cohort of 50 children with schizophrenic parents (index cases) together with 50 matched controls. Half of each group was raised on a kibbutz, and half was raised with their nuclear families in cities and towns in Israel. The children were seen three times: once in 1966-67, when they were about 10-11 years of age, once in 1973 when they were about 17, and most recently in 1981 when they were in their mid-twenties. This last study found significant amounts of psychopathology in the index cases. Aside from several reports of the neurologic findings (suggesting more "soft" signs in the index cases) none of the work has been published before this year. Major progress has been made in the last several years: (1) Major portions of the data have been analyzed; (2) A series of manuscripts have been prepared describing the 1966, 1973, and 1981 studies. These form the basis of a comprehensive report that was published in a single issue of the Schizophrenia Bulletin in 1985. The results of the 1981 study suggest that being raised in a kibbutz environment is more likely to lead to major psychopathology, given a schizophrenic diathesis, than is growing up in the nuclear family within a city environment. Another major follow-up of the Israeli cohort is now in progress, which will include brain CT scans, measures of psychiatric status, behavior on cognitive and attention tests, reanalysis of parental hospital records, reinterviews of the surviving parents, and inspection of the military health records of the subjects.

The objectives of all of these projects are to understand how hereditary and environmental factors interact to make for schizophrenic outcomes of varying types and degrees.

2. Studies on Etiological Factors in Schizophrenia.

Studies of the occurrence of mental illness in families have been useful in identifying familial forms of the illnesses and in the development of hypotheses regarding the form and strength of genetic and environmental factors in etiology. Where these major variables are separated by the process of adoption, specific etiologic hypotheses can be tested separately and in combination. A total national sample of 14,500 adult adoptees in Denmark, including 76 who have developed schizophrenia, provide the basis of one phase of this research; the other phase is represented by schizophrenic patients and their families residing in Roscommon County, Ireland, where the prevalence of schizophrenia appears to be three times higher than its prevalence in England and other Western countries.

F. Cognitive and Perceptual Changes in Affective Illness

This project has involved a variety of studies, all but two of which have been previously completed. Progress over the past year will be summarized below, while more detailed descriptions appear in the individual project reports. Since these projects were under the direction of Dr. Edward Silberman, who has left for Hahnemann Medical College in Philadelphia, no additional data are being collected. The remaining two studies, conducted under Dr. Silberman's direction are summarized below.

a. Psychomotor and psychosensory symptoms in affective illness

Data have been collected on a total of 111 patients with affective illness, partial complex epilepsy, and hypertension (controls). Preliminary analyses indicate that affective and epileptic patients are indistinguishable in frequency of certain kinds of visual, auditory, visceral, and cognitive illusions and hallucination, while paroxysmal motor phenomena are present only in the epileptic group. The relationship of such symptomatology to underlying personality factors and life course of illness in the affective group has now been analyzed and the data are being prepared for publication..

b. Lateralized hemispheric function in depression

Depressed female subjects have been found to show right hemisphere advantage in a verbal processing task which normally displays left hemisphere advantage. Since such a task is an obligatory left hemisphere function in normals, these results suggest shifts of hemispheric function as a concomitant of the depressed state. Further studies involving male depressed subjects and depressed-recovered patients are now being planned. A manuscript has been submitted for publication.

ANNUAL REPORT OF THE LABORATORY OF SOCIO-ENVIRONMENTAL STUDIES
NATIONAL INSTITUTE OF MENTAL HEALTH
October 1, 1985 through September 30, 1986
Carmi Schooler, Ph.D., Acting Chief

At the beginning of this year, Melvin L. Kohn, who had been the chief of LSES for twenty five years, resigned to accept a position in the Department of Sociology of Johns Hopkins University. Although work is continuing steadily along previously initiated lines of research, a change of such magnitude has inevitably led to some shift in the Laboratory's direction. Thus, the research done this year is of two types: that which is a continuation of previous approaches and that which opens up new areas of investigation aimed at explicating the scientific implications of the central findings of the Laboratory's research on the psychological effects of occupational conditions. We begin by briefly discussing the more direct continuations of the Laboratory's previous lines of research and then describe the new research directions.

Much of the directly continued research was carried on in collaboration with Melvin Kohn who maintained his association with the Laboratory in the position of a Guest Researcher. Although the rate of progress was inevitably slowed by Kohn's transition to a teaching position and by the load of his duties as President-Elect of the American Sociological Association, progress was made on several fronts. Kohn and Schooler began the analysis of the intergenerational transmission of the key aspects of psychological functioning in their study of the psychological effects of occupational conditions--intellectual flexibility, self-directedness of orientation, and distress. Kohn, Schooler, and Schoenbach, continued in collaboration with their Polish and Japanese colleagues, their three nation comparison of the psychological effects of social class. Miller, Kohn and Schooler completed final analyses of their study of the relationship between educational self-direction and psychological functioning. They found intriguing differences between their college and secondary school respondents. The experience of self-direction may affect ideational flexibility differently at different stages of educational (and, later occupational) career: more of the effect being direct in at the earlier stages, when the process may be more a matter of cognitive training per se; more of the effect being indirect in the latter stages when the process may become less a matter of cognitive training and more a matter of attitude and adaptability.

Among other lines of research that were continued last year were Schooler's on intra-family dynamics and children's personality development. In addition, Schoenberg and Schooler extended their analysis of the organization and differential psychological effects of several distinct sectors of the United States' economy. They were able to increase the number of industries in their analyses and to link the extensive industrial data collected by the University of Wisconsin sociologists to data collected by the Laboratory of Socio-environmental

Studies on the occupational conditions of individuals. Schoenberg also expanded his methodological inquiries making advances both in the testing of specifications in structural models and in applying such models to categorical data.

Much of this year has been spent developing new, and reorienting old, research approaches so that we can fully understand the scientific implications our research program on the psychological effects of self-directed work and of oppressive working conditions. The central findings of this research are that self-directed work leads to ideational flexibility and to a self-directed orientation to self and society and that oppressive working conditions lead to distress. In our attempt to understand them better, each component of these findings--self-directed work, intellectual flexibility, self-directed orientation, oppressive working conditions and distress--was used as a focus for a new line of research or for a refocus of an older one.

Self Directed Work and Intellectual Flexibility:%

Past research of the Laboratory has clearly established that doing self-directed, substantively complex work leads to greater intellectual flexibility in countries of widely diverse cultures (the United States, Japan, Poland), under a variety of different conditions (paid employment, school work, women's housework) and at different stages in the life span (students, younger workers, older workers). We have not yet, however, delineated the characteristics of the environment or specified the exact cognitive mechanisms through which this process takes place.

One step that has been taken to explain what takes place in environments that foster self-directed activity was Schooler's placing of these findings into the context of a general, if roughhewn, theory of complex environments. This theory grew out of an examination of the research literature of several disciplines including research on children and the aged, as well as social psychological and animal experiments. According to the theory the complexity of an individual's environment is defined by its stimulus and demand characteristics. The more diverse the stimuli, the greater the number of decisions required, and the more ill defined and apparently contradictory the contingencies, the more complex the environment. To the degree that the pattern of reinforcement within such an environment rewards cognitive effort, individuals should be motivated to develop their intellectual capacities and to generalize the resulting cognitive processes to other situations. Much more work, however, is needed in order to operationally define environmental complexity and to model and test the mechanisms through which environmental complexity affects the nature of the individual's cognitive processes. One step in this direction has already been taken. Lines of communication have been opened between the Laboratory and cognitive and life-span psychologists interested in similar problems. As a result of a collaboration with the Division of Behavioral and Social Research of NIA, Schooler is editing, together with Werner Schaie of Pennsylvania State

University, a volume of papers from researchers in the forefront of the attempt to specify the linkages between social structure and cognitive functioning. A post-doctoral Fellow with the appropriate training to collaborate on this line of investigation has been hired and will begin work in September 1986.

Self Directed Orientation:

The finding that self-directed work leads to a self-directed orientation to self and society is a part of the more general question of the determinants of both individual and normative perceptions of individuals' interdependence with and autonomy from the groups to which they belong. Schooler's previous research had indicated that being reared in a complex, multifaceted environment leads to a rejection of external social constraints and a subjective concern for the quality of one's inner life. His recent paper on environmental complexity indicated that, although the evidence is not as extensive as for cognitive functioning, the relevant literature suggests that environmental complexity also leads to a self-directed rather than conformist orientation. In order to see how environmental complexity actually effects social behavior, during the past year Schoenbach and Schooler began an analysis of the socio-environmental determinants of children's affiliative behavior towards their families and friends and of their participation in the social organizations of traditional and youth culture.

Another approach towards understanding the factors underlying the development of a self-directed orientation has been the investigation of how historically determined cultural factors and socio-economically determined social-structural factors affect the nature of the normative and actual relationship of individuals and their social groupings. This long-standing focus of the Laboratory's research started over a quarter of a century ago, when William Caudill began his investigation (completed after his death by Schooler) of the comparative emphasis on interdependence and autonomy in child-rearing practices in the United States and Japan. Japan, as an industrialized society with a quite different, much more group-oriented, tradition of relationships between individuals and groups from that of the industrialized West, is a particularly appropriate place to carry out such an examination of the relative importance of culture and social structure as determinants of autonomy or interdependence. The relative importance of socio-economically determined factors was shown in Naoi and Schooler's recent Japanese replication of the Kohn and Schooler occupational study. Notwithstanding the different emphasis that Japanese culture places on interdependence, in Japan, as in the United States, self-directed work leads to a self-directed orientation to self and society. On the other hand, analyses completed by Schooler and Naoi this year point to the continued importance of tradition in Japan for shaping individuals' assessments of their integration into the social system. They found that Japanese men who work in traditional industries not only have more traditional moral values, but are less alienated and more comfortable with themselves and their position in society than are those who work in industries started after the reopening of Japan to the West in 1854.

During the past year, a study of the way in which traditional culture and social structure interact to affect Japanese women's attitudes towards the interdependence of family members was also begun. An analysis of how these factors affect Japanese women's feelings of responsibility for taking their own and their husbands' aging parents into their households was started. In traditional Japanese culture, taking care of the elderly in this manner, particularly the husband's parents, was seen as a central duty. Initial analyses of the factorial structure of the attitudes of the wives of our Japanese respondents indicate that such attitudes still exist. Our next step will be to examine which social structural-conditions affect the degree to which these traditional, as contrasted to more individualistic or nuclear family based attitudes are held.

This year Schooler also explored through historical analysis how social structure and culture affect the development of individualism in a society. While in Japan on a Japanese Society for the Promotion of Science fellowship, he examined the relationship of the individual to the group in the context of Japanese history. The evidence indicates that Japan underwent a sequence of historical periods similar to the pattern in Europe, during which the level of technical development and the place of the individual in society also roughly paralleled that of similar periods in European history. Furthermore, the emphasis on the importance of the group at the expense of the individual is linked to historical circumstances in Japan remarkably similar to historical circumstances in the West that had similar effects on individualism.

While in Japan, Schooler also continued to work with his Japanese collaborators on the study of the psychological functioning of the children of respondents in their earlier investigation of the effects of occupational conditions on Japanese men. Since the wives of the original respondents have also been interviewed, it should be possible to replicate in Japan the studies the Laboratory has carried out on the intergenerational transmission of values and psychological functioning in the United States. The questionnaire they have developed is not, however, merely a replication of the one used by Kohn and Schooler in the United States. An extensive section has been added examining the nature of the children's view of their relationship to the groups of which they are a part.

Oppressive Working Conditions and Distress:

In the Laboratory's investigation of the relationship between oppressive working conditions and distress, not only did Kohn and Schooler's research demonstrate that oppressive working conditions lead to distress, but earlier Laboratory research by Pearlin and Schooler indicated that although individuals' coping mechanisms can be quite effective when dealing with problems within the close interpersonal role areas of marriage and child rearing, such mechanisms are relatively ineffective when dealing with the impersonal problems found in occupations, many of which are outside of the individual's control. This year Schooler, in collaboration with the Pacific Asian American Mental Health Research Center of the University of Illinois and the Department of Psychiatry of Yonsei University in Seoul began a study in Korea which will make possible the direct examination of the pattern of relationships between occupational conditions and the psychiatric and general medical symptoms of workers and their families in a pre-industrial setting. The study will at the same time lay the groundwork for exploring the nature of the changes occuring in these patterns as modernization occurs. It involves using a version of the occupation questionnaire on a representative sample of husbands and wives who have already been interviewed with the Diagnostic Interview Scheduale (DIS), an NIMH sponsored screening device for finding psychiatric problems in non-hospitalized population samples. The site of the study, Kangwha Island is at present an isolated underdeveloped island, most of whose inhabitants earn their living as small farmers. It is, however, the general expectation that the level of industrialization will increase in the near future. The psychiatric and general medical epidemiology of Kangwha are being intensively studied by the Departments of Psychiatry and Public Health of Yonsei University in Seoul. These departments maintain a research station on the island and have access to relevant medical and government records. This unique combination of circumstances makes it possible to obtain data pertaining to several interrelated and exciting research questions of importance to the LSES. With these data we hope to examine the psychological effects of occupational conditions at a pre-industrial stage of development. Such analyses would also provide a baseline for determining what happens when some of same individuals later go to work in industrial settings. Such research, besides providing us with the possibility of examining the relationship between occupational conditions and psychiatric symptomatology under different levels of modernization, also raises interesting questions about the interrelationships of symptomatolgy, diagnosis, culture and level of socio-economic development. As one way of dealing with these problems, Schooler, in an invited paper to the International Symposium on Psychiatric Epidemiology in Taiwan, outlined a possible statistical interaction based approach to problems of diagnosis and etiology relevant to both cross-cultural, and within-culture studies of mental illness. Schooler and Schoenberg hope to be able to develop this approach further.

We can not, of course, be certain that each of the new or refocussed research directions initiated this year will be fruitful, but we are hopeful that the course of new beginnings and pursuit of previously developed lines of research on which we have embarked will permit the Laboratory of Socio-environmental Studies to continue its high level of achievement.

Annual Report of Laboratory of Cell Biology
National Institute of Mental Health
Michael J. Brownstein, M.D., Ph.D., Chief
October 1, 1985 - September 30, 1986

INTRODUCTION

A number of people have left or will leave the LCB shortly to take jobs elsewhere: T. Reisine, M.-F. Chesselet, G. Rougon, A. Luini, R. Pruss, J. Moskal, D. Kligman, F. Hirata, R. Siegel, M. Brann, J. Wallingford, D. Marshak, and H.-U. Affolter. Their contributions were much appreciated and they will be missed.

Several new investigators have joined the Laboratory this year. Hiroto Okayama and his colleagues have come to the LCB from the Institute of Child Health and Human Development and Russell Martenson, formerly a member of the LCM, has recently changed laboratories as well. S. Lolait, N. Buckley, R. Burch, L. Mahan, and D. Rausch have come to the LCB for postdoctoral training.

In collaboration with the Laboratory of Molecular Genetics, NINCDS, we have established a protein chemistry unit and hope in the next year to identify a unit chief. We look forward to more such space- and money-saving cooperative ventures in the future, among them the establishment of a unit for dishwashing and preparation of culture medium.

The LCB is comprised of people with diverse skills and interests. They can be divided into three groups: neuroanatomists/cell biologists, biochemical pharmacologists/developmental neurobiologists, and molecular biologists. The distinction among the members of these groups is a blurry one; they share interests and collaborate freely. The most senior of the neuroanatomists is Miklos Palkovits who continues to spend half each year in Budapest and half in Bethesda. W. Scott Young has agreed to remain in the LCB as a Senior Staff Fellow and will help both to provide continuity in the neuroanatomy program and to bridge the gap between neuroanatomy and molecular biology. Drs. Zatz and Axelrod continue to oversee strong programs in biochemical pharmacology; Drs. Bonner, Eiden, and Okayama apply molecular biological methods to studies of cellular development and physiology.

Overview

Our goal is to understand the development and function of the nervous system. Our approach, in general, is a reductionist one. We are attempting to understand the molecular mechanisms that underlie specific biological phenomena such as neurotransmitter synthesis and release, neurotransmitter action, phenotypic choice, etc. In particular, we are attempting to isolate, characterize, and study molecules with important roles in the brain and periphery. Examples include peptide neurotransmitters, receptors, factors that regulate neuronal development, and proteins involved in the uptake, storage and synthesis of transmitters. The first step in this process involves development of assays for molecules of interest and using the assays to detect these molecules in the course of their purification. In this way we have recently isolated and characterized a neurite extension factor. Antibodies against the purified factors can be prepared and used for anatomical, biochemical, cell biological, and molecular biological studies; or the purified proteins can be subjected to chemical analyses. Even the partial amino acid sequence of a peptide or protein can be useful to the molecular biologist for isolating DNA complementary to a particular mRNA species. In the last year we have exploited such sequence information to detect and characterize a number of cDNAs including those for chromogranin, substance P, bovine galanin, peptide VQY, and the _raf_ and _A-raf_ gene products. The cDNAs having been sequenced, the structure of the proteins that they encode can be inferred. Furthermore, the cDNAs can be used to isolate their respective genes (and closely related members of gene families), and as probes for studying mRNA production. The latter can be accomplished by means of Northern blotting or _in situ_ hybridization histochemistry.

Peptide/protein isolation and characterization

Drs. Kligman and Marshak have isolated a neurite extension factor (NEF) from the bovine telencephalin and have shown that it is a dimer of the β-subunit of S-100 protein. This glial protein has been known for some time but until now has never had a function assigned to it. NEF may play a pivotal role in CNS development.

Cloning and sequencing of cDNAs and genomic DNAs

Drs. Bonner and Rapp have found a gene that is highly homologous to the _raf_ oncogene. It encodes a 607 amino acid protein, _A-raf_ similar to _raf_ in the kinase domain but different in the presumed regulatory region. Like _raf_, _A-raf_ is a potential oncogene.

Dr. Bonner has completed the cloning of the rat and human substance P genes. He has found, in contrast to published reports, that the substance P message in the bovine striatum typically encodes both substance P and substance K. He and Dr. Affolter have begun to characterize the promoter of the substance P gene.

Ms. Iacangelo in collaboration with Drs. Eiden, Affolter, Grimes, and Herbert, has completed the characterization of chromogranin cDNA isolated from the bovine adrenal medulla. Dr.

Rokaeus has cloned and characterized bovine galanin cDNA. Dr. Koller has isolated porcine peptide VQY cDNA.

Drs. Brownstein, Siegel, Tallman, and Okayama have transfected NIH 3T3 cells (fibroblasts) with a mouse cerebral cortical cDNA library and have isolated a recombinant fibroblast that expresses the central benzodiazepine binding site. They are presently attempting to isolate and characterize the cDNA that encodes this receptor.

Use of cDNA and cRNA for in situ hybridization histochemistry (ISHH)

Drs. Siegel, Young, Zoeller, Brann, Lightman, Palkovits, and Mezey have begun to exploit and have continued to improve the ISHH method this year. They have visualized a number of peptide-, enzyme-, receptor-mRNAs in cells and have detected changes in mRNA levels in response to surgical, physiological, and pharmacological manipulations. Drs. Young and Price are undertaking studies of peptide mRNA levels in autopsy specimens of normal and diseased brains.

Immunocytochemistry

Drs. Young, Siegel, and Palkovits with the help of Ms. Park and Ms. Warden have used immunocytochemistry in concert with ISHH to analyze peptidergic pathways in the CNS and to study peptide producing cells in the periphery. It has proven possible to perform immunocytochemistry and ISHH on the same section.

Studies of receptors

Drs. Brownstein, Siegel, Okayama, Lolait, Buckley, Blalock, and Neal have begun to characterize and use antibodies against the ACTH- and GnRH-receptors to isolate cDNAs that encode them.

Drs. Buckley and Blalock are in the process of raising antibodies that recognize the substance P receptor.

Dr. Lolait has raised an antibody that appears to recognize the renal vasopressin receptor and is characterizing it in detail.

To facilitate the screening of mammalian cell line expression libraries, Drs. Goldstein, Siegel, and Brownstein are in the process of developing a fluorescence-activated colony sorter. This instrument detects and marks colonies of living cells on culture plates that have bound fluorescent ligands.

Studies of peptide and protein production and secretion

Dr. Hook has shown that carboxypeptidase H, one of several proteases required for peptide precursor processing, is synthesized as a molecule with a molecular weight of 80,000. This species seems to give rise to a smaller, active enzyme (MW about 50,000) after it is packaged into secretory granules. It appears that the rate of peptide precursor processing is regulated, in part, by activation of the proenzyme.

Dr. Pruss has developed an antibody against cytochrome b561, a protein which is specifically localized to secretory granule membranes and which has been implicated in ascorbic acid regeneration. The cytochrome appears to be essential for mixed function oxidases such as DBH and the peptide amidating enzyme to function normally. Dr. Pruss plans to purify and partially sequence this cytochrome as a first step toward cloning its cDNA. She hopes ultimately to understand its role in detail and to use it as a probe of secretory granule synthesis.

Ms. Iacangelo, Dr.Eiden and their colleagues are attempting to determine the role of another protein found in storage granules, chromogranin. Having cloned its cDNA (see above) they are raising antibodies against fragments of chromogranin to use for biochemical and anatomical studies.

Drs. Pruss, Eiden, Waschek, Rokaeus, Eskay, Zamir, O'Donohue, Forman, and Mezey have studied peptide expression in primary cultures of bovine chromaffin cells and clonal neuroblastoma cell lines. They have used RIA's to measure peptides (including enkephalin, VIP, galanin, neurotensin, substance P, ANF, and NPY) in the cells and culture medium, immunocytochemistry to estimate the number of cells making any given peptide, and cDNA probes to measure peptide mRNAs. They have shown that 1) there are several subpopulations of chromaffin cells, 2) multiple peptides reside in single cells, 3) the production of any individual peptide is affected by discrete second messengers that may have no effect on other peptides in the same cell, 4) cells can be "recruited" to make peptides under certain circumstances.

Drs. Eiden, Siegel, and Affolter have shown that depolarizing agents cause an increase in the secretion and synthesis of enkephalins in cultured chromaffin cells. Dr. Waschek has demonstrated that the calcium agonist barium can activate enkephalin release and biosynthesis providing evidence that calcium couples secretion and synthesis in the chromaffin cell. In the rat pituitary cell, on the other hand, cyclic AMP seems to couple synthesis and secretion. The biosynthetic process seems to be regulated at the level of transcription and post-translationally. Thus reserpine, which accelerates enkephalin processing in part by increasing the activity of carboxypeptidase H, also elevates the activity of the peptide amidating enzyme.

Biologically active proteins; neurotransmitter actions; second messengers

Dr. Moskal has raised a panel of monoclonal antibodies using neonatal rat hippocampus as immunogen. One antibody has been used in concert with a fluorescence activated cell sorter to analyze, isolate, and maintain in culture a pure population of hippocampal neurons. A second antibody has been found to affect sleep _in vivo_ and long-term potentiation in hippocampal slice preparations. Dr. Moskal hopes to isolate a protein responsible for the actions of this antibody.

Dr. Kligman, in addition to working on NEF, has detected an endogenous modulator (MOP) of C-kinase activity. He has partially purified this protein from bovine brain extracts. It stimulates C-kinase dependent phosphorylation of an 87,000 MW substrate in rat brain synaptosomes. MOP and the protein phosphorylation of which it stimulates remain to be purified and characterized.

Dr. Rausch is attempting to determine the role of oncogene products in neural differentiation. The c-src protein is found in neurons at concentrations higher than in other tissues, a difference that becomes apparent at the onset of neural differentiation. This protein, a tyrosine protein kinase appears to have a different form in neural tissues than it has elsewhere. Dr. Rausch is investigating neuronal c-src and its mRNA. In addition, she is preparing to introduce several oncogenes into PC12 cells. The effect(s) of their expression should provide valuable clues to their roles in neuronal development.

Drs. Eiden, Siegel, and Brenneman have shown that vasoactive intestinal polypeptide (VIP) is secreted by neurons present in cultures of mouse spinal cord and that VIP promotes the survival of non-VIP containing neurons in the culture. This important observation points to a role for VIP in development _in vivo_.

Drs. Burch and Luini have studied receptor-mediated regulation of phospholipases A and C in a rat thyroid cell line. These two enzymes are both activated by α_1-adrenergic agonists but are coupled to the adrenergic receptor by two separate GTP binding proteins. Noradrenaline causes arachidonic acid release and formation of several of its metabolites mainly via the cycloxygenase pathway. The most abundant of these metabolites is PGE_2 which stimulates growth of the thyroid cells. Arachidonic acid is also metabolized via the lipoxygenase pathway to yield an unknown metabolite that stimulates iodine fluxes.

Drs. Burch and Mahan have examined the induction of serotonin N-acetyltransferase activity in the frog retina. The enzyme is altered by agents which activate or inactivate certain GTP-binding proteins and by agents which affect arachidonic acid metabolism.

Dr. Jelsema is studying the role of GTP-binding proteins in modulating phospholipase A_2 and C in rod outer segments of the bovine retina. G proteins appear to be involved in both activation and inhibition of the phospholipases. Transducin, the major G protein in the rod outer segment participates in activating phospholipase A_2 and C. The $\beta\gamma$ subunit of transducin activate phospholipase A_2; the phosphorylated form of the subunit has been implicated in phospholipase C activation.

Drs. Jelsema, Axelrod, Burch, and Mahan have investigated the interaction of various neurotransmitters with the phospholipases of the rod outer segment of bovine and frog retina. Activation of D_2 dopamine and somatostatin receptors stimulate phospholipases, an effect which may be G protein mediated.

Chronobiology

Drs. Zatz and Moskal have developed a rapid and specific extraction assay for ^{3}H-melatonin formed from ^{3}H-tryptophan. The assay has been used to study cyclic production of melatonin by dissociated chick pinealocytes in culture. A rhythm in melatonin synthesis persists for three cycles in constant red light. Alternating red and white light or red light and darkness can drive the melatonin rhythm for five or more cycles. Cyclic administration of potassium, norepinephrine, or forskolin also entrain the rhythm. Thus, while the true free-running rhythm cannot be studied in cultured cells, entrainment can.

Drs. Zatz and Wallingford have been trying to identify and characterize the pineal photopigment mediating entrainment. Their results suggest that this photopigment may be different from those mediating vision.

Annual Report of the Laboratory of Cerebral Metabolism
National Institute of Mental Health

October 1, 1985 through September 30, 1986

The major changes in organization introduced into the Laboratory last year have been implemented, but operational adaptation to these changes are still in progress. In particular, the Section on Clinical Brain Imaging (Robert Cohen, Chief) has been added, and the previous Section on Myelin Chemistry under Dr. Kies has been abolished. These changes have imposed some new problems regarding spatial arrangements and personnel assignments which are still in the process of being resolved to achieve optimal modes of operation. Specifically, the Section on Clinical Brain Imaging is physically located in Building 10, whereas the remainder of the Laboratory is in Building #36. This distance is an effective deterrent to ideal interaction, communication, and collaboration, but attempts to integrate some of the activities and to develop collaborative projects are under consideration.

The abolition of the Section on Myelin Chemistry has been associated with greater problems. This change was associated with the loss of personnel, space, and some resources that this Section had previously contributed to the operation of the Laboratory as a whole. Russell Martenson, a tenured biochemist for a number of years, has been transferred out of the Laboratory but still utilizes 1-1/2 modules belonging to our Laboratory. Another module was transferred to another laboratory as well. Dr. Marian Kies and Gladys Deibler still remain, but their research activities have been curtailed and are now limited to a new project just being initiated on proteinase activities in brain and their role in the regulation and control of protein neuropeptide transmitter turnover in brain. Dr. Bernard Driscoll, previously of the Section on Myelin Chemistry, is now developing a new program of research that in the next few year's should become a major nucleus for other research projects in the Laboratory.

Section on Developmental Neurochemistry
Louis Sokoloff, Chief

The major efforts of this Section last year to examine the validity of the criticisms of the deoxyglucose method have been completed this year, except for the publication of the final major manuscript derived from this work. G. Dienel and T. Nelson have carried out a number of experiments initiated last year and completed this year that proved that the criticisms were without foundation. Dienel has systematically repeated the studies on which the criticisms were based, but with careful examination at each step of the validity of the conclusions. He has fully explained the results from which the erroneous conclusions were drawn and found that the critics had failed to carry out proper controls and to recognize that their results were reflections of radioactive contaminants and not the compounds they thought they were. This work is now in a manuscript draft and should be submitted for publication within the next few months.

Drs. K. Mori, G. Dienel, T. Nelson, T. Jay, and Ms. N. Cruz have finally initiated the studies to adapt the deoxyglucose method for use in pathological tissues. This requires the determination of local lumped constants. A theoretical basis for determination of local lumped constants on the basis of measurement of local 3-O-methylglucose tissue:plasma distribution ratios has been developed. Studies are in progress, first to define the limits of 3-O-methylglucose metabolism in brain, if any, and secondly, to relate the brain:plasma distribution ratio to the value of the lumped constant.

L. Porrino, now of NIDA but still a Guest Researcher in our Laboratory, has continued her collaborations with I. Kopin of the NINCDS and with Dr. E. Palombo and V. Ho of our Laboratory on the effects of MPTP-induced Parkinsonism on local cerebral glucose utilization and the changes produced by DOPA therapy. They had previously found numerous localized decreases in glucose utilization in the brain in MPTP-induced Parkinsonism. These changes were primarily in the basal ganglia and were reversed everywhere, except in the substantia nigra compacta, by DOPA treatment. They have now been studying the effects of acute MPTP administration before the Parkinsonism syndrome develops and found marked increases in glucose utilization in the substantia nigra compacta. It appears that the drug first markedly activates metabolism in this structure, which eventually degenerates with cell death leading to Parkinsonism.

Dr. C. Smith and N. Eng, after a long period of methodological development, have completed two critical experiments necessary for further progress in the development of the method for measurement of local cerebral protein synthesis. These results indicate that there is approximately 20% recycling of amino acid derived from the slow but steady rate of protein degradation back into the precursor pool of amino acids for protein synthesis. This is the first time this measurement has ever been made *in vivo*.

Drs. E. Kaufman and T. Nelson have been completing their studies of the synthesis and metabolic degradation of γ-hydroxybutyrate, a compound normally present in brain in small amounts but which has profound biochemical electrophysiological, and behavioral effects in pharmacological doses. They have delineated parts of the metabolic pathways for synthesis and metabolism of this compound and are in the process of preparing a number of manuscripts on this work before irreversibly terminating the project.

Dr. B. Driscoll has initiated a program to develop a cell culture facility which will provide basic material for major new Laboratory projects planned for the future. He has succeeded in establishing culture procedures in chemically defined media for primary cultures of neurons from fetal brain destined to become dopaminergic cells and dopamine-receptive cells. He has found co-cultures of the two types of cells improves their growth and neurite production.

Dr. M. Kies and Ms. G. Deibler are bringing their research on myelin basic protein to completion by the end of this fiscal year and have initiated a project on the study of proteinases in brain which may play a role either in the post-translational processing of the precursor proteins that provide neuropeptide transmitters in brain or in the termination of action of these peptide neurotransmitters.

Section on Clinical Brain Imaging

Robert M. Cohen, Chief

This Section, a new addition to the Laboratory, is dedicated to the development, refinement, and use of methods employing PET for studies of neuropsychiatric disorders. They have been actively pursuing these goals. Dr. R. Cohen and his group have been developing statistical principles for analyses of the enormous amount of data derived from PET studies to evaluate the significance of generally small changes in the measured functions. They have continued their studies on the effects of somatosensory stimulation on local cerebral glucose utilization in normal and schizophrenic patients, both on and off neuroleptic therapy. Their results provide some support for the existence of "hypofrontality" in schizophrenic brains.

Dr. C.C. Chiueh has been continuing his studies on the use of L-6-^{18}F-dopa to map the distribution of dopamine in human brains *in vivo* by PET. He has tested the possibility to do so in the primate model of Parkinsonism induced by MPTP and confirmed by this technique the expected depletion of Dopamine in the striatum in this condition. These preliminary studies provide encouragement for the development of a useful method to study the distribution of a key neurotransmitter in human brain.

Annual Report of the
Laboratory of General and Comparative Biochemistry
National Institute of Mental Health
October 1, 1985 to September 30, 1986
Giulio L. Cantoni, M.D., Chief

Fiscal year 1986 has been a very productive and successful year for the LGCB. No major administrative problem have arisen and we have been able to operate efficiently within the budgetary guidelines imposed on us by the fiscal restraints.

The research program of Dr. Cantoni's group continues to revolve around projects that are closely interrelated and all deal with the regulation of biological methylation reactions and their significance for various physiological processes. Our research group is small and all the members of the staff work together as a team participating in one way or another in all the different projects since these are closely interrelated technically as well as conceptually. Individual contributions are recognized appropriately by authorship, meeting presentation, etc.

The objectives and principal results of the main projects under investigation are summarized below while technical details are described more fully in the individual project reports.

Our approach, as in previous years has proceeeded along two lines of research:

A) in vitro we pursue our long standing interest in the basic enzymology and structure of enzymes that utilize Adenosyl homocysteine (AdoHcy) and/or Adenosylmethionine (ADoMet).

1) For several years we have been developing experimental evidence to support the hypothesis that the ratio of AdoMet/AdoHcy is one of the principal determinants for the utilization of AdoMet in various biochemical and biological systems. As the result of our work, as well as that of others it is now well established that S-Adenosylhomocysteine-hydrolase plays a critical role in modulating the AdoMet/AdoHcy ratio in cells. We have used several approaches to determine the structure and function of this enzyme and in the last year have made considerable further progress in this endeavor:

a) Determination to the primary structure of the enzyme has been approached in two ways: by isolation of peptides generated from partial fragmentation of the enzyme and determination of the corresponding amino acid sequences, and by characterization of a cDNA clone isolated from cDNA rat library by use of antibodies. The secondary structure of the enzyme in its active and inactive form has been examined by means of fluorescence spectra, circular dichroism and photoaffinity labeling.

b) AdoHcyase is an enzyme of unexpected complexity: it consists of a variable number of different but identical subunits (two for the plant enzyme, four for the mammalian enzyme and six for the bacterial enzyme) and each subunit binds tightly one mole of NAD. The reaction catalyzed the enzyme involves an oxidation-reduction cycle of enzyme-bound NAD. We found that incubation with

K^+, Mg^{++} and ATP leads to inactivation of the enzyme with loss of enzyme-bound NAD. The inactive form of the enzyme binds cAMP and can be reactivated upon incubation with NAD with displacement of cAMP. It seems therefore that NAD and cAMP bind to the same or similar sites on the enzyme. The reversible inactivation cycle has been studied in vitro and further experiments will be required to determine whether these reactions have a physiological counterpart.

c) The purified enzyme has been used to immunize rabbits and produce antibodies. The antibodies thus obtained have been used, as noted above, to screen a cDNA library from rat liver and select a clone or clones, containing the complete nucleotide sequence of the protein. The antibody against the enzyme is also being used to develop a radioimmunoassay (RIA) for the enzyme. RIA will be used to measure the turnover of the enzyme and its level in different types of cells or under different physiological conditions (differentiation, hormonal stimulation, etc.)

d) We have continued examination of a number of adenosine analogs that can function as substrates and/or inhibitors of AdoHcyase. Using these inhibitors we have been able to manipulate in various ways the intracellular levels of AdoHcy or its congeners (PurHcy). Both AdoHcy and PurHcy are potent inhibitors of the enzymes that utilize AdoMet. By correlating the intracellular levels of AdoMet and AdoHcy or its congeners we have obtained direct evidence in support of the working hypothesis that has guided our work for several years namely we have determined that different methyl-pherases are inhibited to a different degree by the same level of AdoHcy (or PuHcy). It has been therefore established that there exists a biological hierarchy for the utilization of AdoMet for transmethylation reactions and that in the absence of other regulatory factors the intracelluair AdoMet/AdoHcy ratio plays a regulatory role in biological methylations in vivo.

B) in vivo we analyze the role of biological methylation reactions in various model systems such as 1) cellular differentiation and 2) macrophage chemotaxis.

1) During cellular differentiation the degree of DNA methylation is known to undergo changes and to be inversely related to gene expression but no mechanism has been described to account for the transient demethylation observed during cellular differentiation. Cantoni, in collaboration with Dr. A. Razin have investigated this problem and discovered a novel enzymatic mechanism which in the absence of DNA replication, brings about a modification in the pattern of DNA methylation by the specific removal of methyl cytidine (mC) residues and their replacements by cytosine.

The scientific background of this project may be briefly reviewed: in eukaryotes the pattern of DNA methylation is clonally inherited and tissue specific; moreover a wealth of experimental data has accumulated over the past few years indicating that 1) the pattern of DNA methylation undergoes significant changes during differentiation and 2) expression of tissue specific genes is inversely related to the degree of methylation of the corresponding genes. meC is the only methylated base found in eukaryote DNA where it is located exclusively in the sequence mCpG. The synthesis of mC occurs as a post replicational modification of DNA by transfer of the methyl group of AdoMet to unmethylated CpG sites in DNA. It is thought that the clonal inheritance of methylated pattern is due to an enzyme known as maintenance methylase, that acts in conjunction or immediately after DNA replication by DNA polymerase. Nothing was known about the mechanism underlying changes in the pattern of DNA methylation a phenomenon that clearly must involve both demethylation and remethylation reactions. FELC can be induced to differentiate in tissue culture by treatment with appropriate chemical agents, and it has been observed that treatment of FELC with these inducers results in transient genome-wide demethylation.

On the basis of these earlier observations we decided to investigate the mechanism by which active demethylation might take place. We concluded first of all that in FELC the kinetics of the demethylation process are not consistent with a passive mechanism such as would result from the inhibition of maintenance methylase during DNA replication. Next we reasoned that a mechanism involving direct demethylation of DNA through removal of the methyl group of mCytidine could be excluded a priori since such a mechanism would require a biochemically unprecedented cleavage of a C-C bond. This important conclusion stemmed directly from Cantoni's earlier studies on the biochemical mechanisms in transmethylation, and led us to postulate the existence of a novel mechanism whereby in the absence of DNA replication, 5-methyl cytosine residues in DNA would be replaced by cytosines. Having formulated this working hypothesis we proceeded to devise the experimental strategy required to test its validity.

Our findings may be summarized as follows: after separation of newly replicated DNA from parental DNA by isopycnic centrifugation we could demonstrate conclusively that the genome-wide DNA demethylation induced in FELC by HMBA is associated with the specific incorporation of deoxycytidine into non-replicated (parental) DNA; we have shown furthermore that this demethylation is transient and that the newly incorporated deoxycytidines residues are located in CpG sequences since they can be remethylated thereby generating a new and different pattern of DNA methylation. These findings must be regarded as a major discovery that will open the way to a number of interesting new studies. It will be of interest first of all to determine if this mechanism undelies the differentiation if myoblasts into myofibers, a system that has been used in this and other labs as a model for cellular differentiation. It will also be interesting to investigate the intimate nature and control of the biochemical mechanism responsible for mC replacement, and to attempt the characterization of the remethylation system. It is reasonable to suppose that the remethylation reaction will involve the de novo methylase, an enzyme system that has hitherto been inadequately characterized and furthermore we can anticipate that the remethylation reaction will be inhibited by changes in the intracellular ratio of AdoMet: AdoHcy or its congener DZA HCY. Thus it will be particularly interesting to determine the effects of methylation inhibitors on FELC differentiation.

2) Macrophage chemotaxis may be considered as a model of behavioral response to sensory stimuli or more generally of cell to cell communication.

The important discovery in this laboratory that chemotaxis by a macrophage cell line is specifically inhibited by 3-deaza-AdoHcy has allowed us to search chemical reaction(s) that would show similar inhibitor specificity and assess the significance of certain biochemical reactions in macrophage chemotaxis.

We concluded that reactions catalyzing the synthesis of phosphatidylcholine by methylation of phosphatidylethanolamine, the release of arachidonic acid when cells are incubated with a chemo-attractant, methylation of the lysine and arginine residues of protein, and protein carboxymethylation are not required for chemotaxis by RAW264 cells since they were inhibited non specifically by a variety of compounds.

In contrast, the synthesis of a small number of proteins, identified after separation by two-dimentional polyacrylamide gel electrophoresis, showed the necessary inhibitor specificity for involvement in RAW264 chemotaxis. Quantitation of 100 of the more prominent proteins on the gels by computerized densitometry showed that in cells treated with 3-deazaadenosine the synthesis of approximately 10% of the proteins was specifically inhibited by more than 50%. The correlation of strengthened by the finding that other inhibitors of chemotaxis inhibited the synthesis of the same subset of proteins. A common feature of the inhibitors of chemotaxis described above is that they all can inhibit the synthesis of functional mRNA.

As a working hypothesis we have suggested that treatment of RAW264 cells with 3-deazaadenosine, 3'-deoxyadenosine, and the combination of EHNA, adenosine and homocysteine inhibits the synthesis of functional mRNA coding for one or more chemotactic proteins. In support of this hypothesis, we have found that 3-deazaadenosine is a more potent inhititor of the synthesis of polyadenylated mRNA than 3-deazaaristeromycin and that AdoHcy and 3-deazaAdoHcy do not inhibit in vitro translation.

A detailed examination of the effects of 3-DZA on attractant binding and further insight into the steps required for signal transmission has been hindered by an interesting circumstance: RAW264 and other chemo-responsive cells that grow in culture, respond only to attractants that are complex molecular mixtures with multiple biological activities, whereas cells such as human or rabbit leukocytes that respond to FMLP (N-formylmet-leu-phe) and other chemically defined attractants do not grow in culture. To overcome this impasse we proceeded to construct a hybrid cell line by fusion between human leukocytes and thioguanine resistant RAW264 cells. A hybrid cell line (WBC264-9) which exhibits chemotaxis to FMLP and other peptides has been characterized and patented.

A study of the binding of radiolabeled FMLP to WBC264-9 cell indicated that WBC264-9 cells contained approximately 1200 receptors per cell, compared to reported values from 2000 to 120,000 per cell for human leukocytes. The apparent dissociation constant for FMLP binding to WBC264-9 membranes was 2 nM, in agreement with values reported for the high affinity site of human leukocytes. It has been proposed that the high affinity receptors for FMLP mediate chemotaxis.

Studies on the human FMLP receptor has been carried out in collaboration with Dr. L. Harvath. It was found that the FMLP receptor complex for chemotaxis transduction mechanism is different in human neutrophils and monocytes.

In collaboration with Dr. Harvath, we have also developed flow cytometric procedures that allow us to determine the subpopulation of leukocytes in whole blood that bind a fluorescent FMLP derivative and to determine the rate of binding of fluorescent FMLP to human neutrophils.

In addition to chemotactic receptors, one or more guanine nucleotide proteins (N-proteins) are required for chemotaxis by RAW264 and WBC264-9 cells. This conclusion is based on the observation that chemotaxis of either RAW264 or WBC264-9 cells is inhibited upon incubation of the cells with either cholera toxin or pertussis toxin. In all cases entry of the toxin is required and there is a correlation between toxin-catalyzed ADP-ribosylation of an N-protein and the inhibition of chemotaxis.

In agreement with observations in several other laboratories, we found that the major membrane protein ADP-ribosylated by cholera toxin is distinct from that ADP-ribosylated by pertussis toxin.

Although both cholera toxin and pertussis toxin can affect cAMP levels, our evidence indicates that cAMP is not involved in chemotaxis. This was shown by elevating cAMP with either isoproterenol or forkolin to levels comparable to those achieved with cholera toxin. Chemotaxis of cells treated with isoproterenol or forkolin was not inhibited, showing that increased levels of cAMP *per se* do not inhibit chemotaxis.

The Section on Alkaloid Biosynthesis continue their investigations in two major areas:

(1) The aspartate family of amino acids and its regulation. Our studies in this area are now sufficiently complete so that one may begin to glimpse the major regulatory features which govern the operation of this complicated, multi-branched biosynthetic pathway. While additional work is needed to answer remaining uncertainties, we think we are almost at the point at which one can make rational suggestions for genetic engineering steps which have a realistic chance of increasing the dietary availability of both lysine and methionine, the limiting protein-derived factors in most diets world-wide which are poor in animal proteins.

(2) Phospholipid biosynthesis: Following upon our unexpected discovery that in *Lemna* the methylation steps involved in phosphatidylcholine synthesis occur at the phosphobase level, not the phosphatidylbase level ($\geq$ 99:1), we have now (a) demonstrated the enzyme(s) that catalyze the requisite methyltransfers; (b) investigated the earlier steps in this pathway to determine whether ethanolamine moieties are formed by decarboxylation of phosphoserine, of serine, or of phosphatidylserine. The latter pathway, the one favored by most current reviewers of the existing literature, has been shown to be non-contributory. The final choice between phosphoserine and serine awaits the results of samples now in hand; (c) demonstrated that ethanolamine formation (in the general sense) and phosphoethanolamine methylation (the critical branch points leading to phosphatidyl-ethanolamine *plus* phosphatidylcholine and to phosphatidylcholine, respectively) are under exquisitely sensitive regulation by ethanolamine and choline, respectively; and (d) taken the first steps in exploring the generality of these phenomona in the plant kingdom. To the extent that time permits we intend to follow up these novel insights and, in possible, determine the extent to which they are applicable, also, to mammalian phospholipid metabolism.

Prof. A. Razin returned to NIH to complete his term as Fogarty Scholar and was the guest of the LGCB during this period. During his visit he collaborated with Dr. Cantoni and his colleagues in the project described above. Dr. Cantoni was invited by The Hebrew University in Jerusalem to visit the Hadassah Medical School: this visit took place during the month of June 1986. Dr. Cantoni gave two lectures at the Hebrew University and discussed ongoing research with Profs. Razin, Cedar, Levitzy and others.

During his visit to Israel Dr. Cantoni was also invited to lecture at the Weizmann Institute of Science in Rehovot, where he met with Profs. Yavin, Yaffe, Patchornik, and Kam. In Jerusalem Dr. Cantoni was the guest of the city of Jerusalem and lodged in the exclusive guest house Mischkenot Shenanim.

Drs. Cantoni and Razin were invited to organize a meeting on Cellular Differentiation and DNA methylation under the sponsorpship of the FEBS, and plans are now underway to this effect. It is anticipated that this international FEBS COURSE will take place in June, 1987 in Crete.

Dr. Cantoni also was invited to lecture at the University of Paris, Jaques Monod Institute of Biologie Moleculare and visited there with Prof. G. Bernardi, Grunberg-Monago and Truffa Bachi. Dr. Cantoni was invited to deliver the annual Sigma Xi lecture in Memphis, Tennessee and also lectured at the U. of Tennessee, Dept. of Biological Chemistry. Finally, Dr. Cantoni was invited by the Italian National Research Council to attend the "Science Week" in Rome, Italy where various aspects of the international collaboration between Italy and other countries were examined in detail.

Drs. Aksamit, Backlund, Giovanelli, Mudd and Unson participated in the annual meeting of the Society of Biological Chemists and presented posters at this meeting.

Annual Report of the Laboratory of Molecular Biology
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Howard A. Nash, M.D., Ph.D., Chief

Introduction

The Laboratory of Molecular Biology was formed in 1984 by the conjoining of three independent research programs. The experience of the past year has made it clear that the laboratory functions well as a coherent, interactive unit. Not only has each section made significant progress toward its research goals but each has contributed in an important way to the scientific work of the other groups within the laboratory. Each section continues to be a small operation consisting of a chief, one or two technicians, and two or three post-doctoral fellows. The small size promotes direct scientific contact between senior and junior staff and encourages scientific exchanges both within the laboratory and with colleagues elsewhere.

Current progress in the individual sections will be outlined in the summaries of research that are presented below. The Section on Regulatory Proteins, headed by Dr. Werner A. Klee, has studied the way receptors couple external signals to the internal milieu of the cell. A receptor for opiates has been the continuing focus of this work and recent studies have also examined a receptor for bradykinin. The Section on Molecular Genetics, headed by Dr. Howard A. Nash, has concentrated its efforts on the mechanism of the reaction that inserts the DNA of a virus into the chromosome of its host. A recent initiative in this section is targeted at the identification of genes encoding components of the nervous system of the fruit fly. The Section on Biophysical Chemistry, headed by Dr. David M. Neville, Jr., has studied the way in which proteins, epitomized by protein toxins, are transported across and into cell membranes. Knowledge about the toxins has been used to construct potent and selective immunotoxins reagents.

SECTION ON REGULATORY PROTEINS

Werner A. Klee, Ph.D., Chief

The Section on Regulatory Proteins is engaged in a program aimed at understanding the mechanism of signal transduction across cell membranes. Receptors on the cell surface, such as those for the opiates, are coupled to enzymes, such as adenylate cyclase, on the inside of the membrane via the mediation of GTP-binding regulatory proteins (G-proteins). In this manner, information is transmitted to a cell from neighboring cells and from the fluid environment. The goal of the work is to dissect this system into its parts, and study each of the components of the system both in isolation and as a reconstituted functional entity.

The work of the section has for several years been concentrated upon opiate receptors in the cultured neuronal cell line, NG108-15. These cells are richly endowed with opiate receptors of a single type, and are therefore an ideal system in which to study their properties. The receptors are coupled, as inhibitors, to adenylate cyclase both in these cells and in brain tissue. Activation of the receptors with opiates or opioid peptides reduces cellular cyclic AMP levels and thereby lowers the extent of phosphorylation of many cellular enzymes. In analogy to the addictive process, the cells become tolerant to and dependent upon opiates after prolonged exposure. This adaptive process is due to a gradual increase in adenylate cyclase activity which serves to maintain normal cyclic AMP levels in the continued presence of opiates. With opiates such as morphine, adaptation occurs in the absence of changes in receptor number. Other opioids, such as the enkephalins produce receptor down-regulation as well as increased adenylate cyclase activity upon chronic exposure.

The NG108-15 cells also contain a number of other receptors for neuropeptides and neurotransmitters. Among the more interesting of these receptors is that for the peptide bradykinin, which is coupled to the activation of phospholipase C in the cells. Thus, activation of bradykinin receptors elevates cellular levels of diacylglycerol and Ca^{2+} and thereby stimulates the phosphorylation of a class of cellular proteins. The presence of these receptors has supplied an opportunity to compare the mechanisms by which receptors are coupled to very different effector systems.

The group has, over the past few years, developed procedures for the solubilization of receptors from membranes by means of extraction with the zwitterionic detergent CHAPS and has isolated affinity labeled opiate receptors in a homogeneous state. Such receptors, because of presence of covalently linked opiates are more useful for structural than for functional studies. Receptor preparations which have not been irreversibly modified are needed for functional reconstitution studies. To this end, several affinity columns, consisting of opiates covalently linked to cross-linked agarose beads, have been prepared to which opiate receptors bind. In combination with lectin affinity chromatography this type of proceedure has allowed the partial purification of opiate receptors from NG108-15 cell membranes. The yields obtained in these procedures have, so far, been low when receptors are assayed by opiate binding activity. In order to ensure that analytical methods are adequate for quantitating functionally active receptors, and as a goal in itself, a major effort has been made in the past year to develop a chemically defined reconstitution system.

Adenylate cyclase has been purified from frozen rat brain by a combination of lectin and either calmodulin or forskolin affinity steps. The preparation is accomplished in one day and the enzyme so prepared, while not completely homogeneous, is highly purified and free of all regulatory proteins. The regulatory proteins, Gs and Gi, have been purified from rabbit liver by a modification of published procedures. These proteins have been reconstituted in liposome vesicles from CHAPS solution by dialysis in the presence of phospholipids. In the reconstituted vesicles, adenylate cyclase activity is stimulated 4-5 fold by Gs. The stimulated, but not the basal, activity is inhibited by Gi. Interestingly, these effects of the G proteins do not require the addition of GTP. This unexpected finding is apparantly a reflection of the fact that the purified G proteins are associated with tightly bound GDP and therefore are partially active in the absence of added guanine nucleotides. Efforts are currently underway to remove the bound guanine nucleotides from Gi in odrer to render the reconstituted system sensitive to GTP for inhibition of adenylate cyclase. It will then be possible to assay opiate receptor activity in this defined system and use the assay both as a tool for monitoring receptor purification and for clarifying the mechanism of receptor-effector coupling.

Several GTP-binding regulatory proteins have been characterized, but, with the exception of that of Gs, the functions of these proteins have not been firmly established. The G proteins are members of a closely related family and are each composed of three subunits: α, β , and γ. The major differences in structure among the several G proteins are found in the α subunits. The β subunits are all identical or nearly so and the β–γ subunit complexes of the most diverse of the G proteins have been found to substitute for one another. Two such proteins, Gi and Go, were purified from bovine brain and used to prepare antibodies which recognize the α subunits of one or the other protein. With the help of these antibodies the amounts of these proteins in brain, NG108-15 cells, and several other tissues could be accurately determined. Go is a highly abundant brain constituent which accounts for 1-2% of the total membrane protein; the levels of Gi are significantly lower. During neo-natal development of the rat brain, Gi and Go levels rise by a factor of approximately three. A novel G protein which is neither Gi, Go nor Gs has been discovered to be the major pertussis toxin substrate of rat C6 glioma cells and human neutrophils. The functional significance of the new G protein is unclear because of the fact that an intensive search has failed to find a receptor to which it is coupled in C6 cells. Isolation of the protein is planned to study its properties in reconstituted systems of receptors and effector enzymes.

Thus, there are at least three distinct G proteins which are pertussis toxin substrates, namely Gi, Go, and the C6 and neutrophil protein, (as yet unnamed). One or more of these proteins must mediate receptor coupled inhibition of adenylate cyclase. One or more of these proteins also must mediate at least some instances of receptor coupling to phospholipase C activation. Data obtained so far are most consistent with the hypothesis that Gi mediates opiate receptor coupled inhibition of adenylate cyclase and Go mediates bradykinin receptor coupled activation of phospholipase C. This hypothesis requires further testing, particularly in light of the observation that NG108-15 cells contain predominantly Go and that the amount of Gi present is less than the amount of opiate receptors present. The possibility that different G proteins may carry out similar functions in different cells or even the same cell cannot be ruled out. Thus, specificity of hormone response may be determined primarily by the receptor and not by the nature of the particular coupling proteins present in the cell.

SECTION ON MOLECULAR GENETICS

Howard A. Nash, M. D., Ph.D., Chief

The Mechanism of Site-Specific Genetic Recombination

The way in which the DNA of a bacterial virus recombines with the DNA of its host has been the focus of the major research effort in the Section on Molecular Genetics. Integrative recombination of bacteriophage lambda is a paradigm for related reactions such as the integration of retroviruses and the rearrangements of the genes for T-cell receptors. This laboratory has established the lambda integration reaction in a cell-free system and has thus been able to analyze the components of this biological process. Our current objectives are to understand the way in which recombination proteins contribute to the specificity and effeciency of the reaction, the way in which the state of the viral DNA governs its reactivity, and the way in which viral and host DNA sequences recognize each other with precision.

Previous work from this laboratory has shown that only two proteins play a direct role in integrative recombination. One, called Integrase (or Int), is encoded by the virus and contains the catalytic breakage and reunion activity needed for the exchange of DNA strands. The other, Integration Host Factor (IHF), is encoded by E. coli and plays an important but accessory role in recombination. IHF binds to three specific sites within attP, the segment of viral DNA that is necessary and sufficient for viral integration. We have altered the DNA sequence of each of the three IHF binding sites in attP to test whether each is required during recombination.

Four base pair alterations were introduced into each of the IHF binding sites in attP. In each case, recombination in vitro falls dramatically, proving for the first time that all three IHF binding sites are needed for attP function. Although each mutated site is also depressed for recombination in vivo, mutations in two of the three sites had a much smaller effect in vivo than in vitro. This suggests that another factor(s) in E. coli can replace the requirement for specific IHF binding at these two sites. Not only does this finding provide an assay for the putative factor, it also provides evidence that the three IHF binding sites play at least two distinct roles, one of which can be filled only by IHF.

The topological state of attP determines its reactivity; under physiological conditions attP needs to be on a supercoiled circle in order to recombine efficiently with the host DNA. To determine if supercoiling affects the way in which recombination proteins bind to attP, we have extended the methods of DNA footprinting to permit the probing of specific protein-nucleic acid interactions on supercoiled as well as on linear templates. Our new method combines attack on DNA by dimethylsulfate (DMS) with indirect end-labeling. With this method we have observed for the first time that supercoiling alters the affinity with which Int binds to two of its sites in attP. Interestingly, the effect of supercoiling on Int binding requires that IHF be present, suggesting a multi-protein interaction. Moreover, for supercoiling to have an effect on one Int binding site, other Int binding sites located about 200 base pairs away, must be present. Taken together, our data point to the existence of a higher-order structure for attP in which several proteins bound to distinct segments of DNA interact with one another. Supercoiling must stimulate recombination not by favoring the binding of individual proteins in this array but by enhancing the wrapping of the nucleoprotein assembly. This conclusion, which agrees with our earlier topological results, is proven by our finding that under normal recombination conditions all the Int and IHF binding sites of linear attP are filled with protein; the linear array is inactive because it is not wrapped. We have shown that the degree and sign of supercoiling needed to promote wrapping (as detected by enhanced affinity for Int) correlates well with that needed to promote recombination. This leads us to believe that we have at last uncovered the primary role for supercoiling in lambda integrative recombination.

Recombination between attP and its counterpart in the bacterial host, attB, requires that a particular segment of the two sites consist of identical DNA sequences. The exact sequence in this so-called overlap region is not critical; a wide variety of alterations from the naturally occuring sequence are tolerated if the identical changes are made in the partner site. An important goal in understanding how recombination works is to determine how homology in the seven base pair overlap region governs the reaction. We have now shown that the initial exchange of one pair of strands between attP and attB is not blocked when homology in the overlap region is disrupted but that, under these conditions, recombination can not be completed by the exchange of the second pair of strands. Our experiments depend on our capacity to detect intermediates in recombination in which only one pair of strands has exchanged. Such intermediates, called Holliday structures, have often been postulated but have never been observed to accumulate during in vitro recombination. We succeeded in detecting these structures by using an artifically constructed attB which contains a phosphorothioate analog in place of a phosphodiester at the point of Int cleavage in one strand. As described for many other enzymes, the phosphorothioate analog is poorly attacked by Int. As a result the attB analog is depressed for recombination but accumulates a novel species during recombination reactions; analysis of the strand composition of the novel species demonstrates that it contains a Holliday junction. We find that the accumulation of Holliday structures in crosses with phosphorothioate-containing attB depends upon many parameters known to affect standard recombination reactions: supercoiling of attP, presence of IHF, correct spacing of Int binding sites in attP, etc. However, we find that altering a total of three out of seven base pairs in the overlap region decreases accumulation of Holliday structures by no more than a factor of three. In contrast, completed recombinants are decreased by at least a factor of 100. We conclude that homology is not essential for synapsis, thus eliminating our previous hypothesis. Instead, our data show that homology is essential for a process that follows the first strand exchange.

Genetic Neurobiology of Drosophila

The goal of a project that is in its infancy within the Section of Molecular Genetics is the isolation of mutations that affect the nervous system. We seek to apply a combination of pharmacologic and genetic tools to the components of synaptic transmission in the fruit fly, Drosophila melanogaster. In this way we hope to isolate mutations in the genes encoding important components of the synaptic machinery and its regulatory apparatus. Much of our effort has been devoted to defining the potency of candidate pharmacologic agents and to establishing protocols for chemical mutagenesis of Drosophila.

In many invertebrates, formamidine insecticides appear to alter behavior by acting as agonists of octopamine. Although formamidines are not generally effective in Dipterans, we have that found that amitraz, a commercially available formamidine, does alter the behavior of Drosophila. The effect is large enough to be the basis for a mutant screen. Unfortunately, we have been unable to find agents in the formamidine family that alter behavior more potently. Moreover, we could not demonstrate synergy between octopamine (either fed or topically applied to Drosophila) and amitraz; indeed a weak antagonism was discovered. We think therefore it is prudent to delay use of formamidines as selective agents until we locate a more potent agent and/or demonstrate more convincingly that amitraz affects behavior via the octopamine pathway.

The cellular and molecular targets of general anesthetics have been the subject of theory and experiments for almost 100 years. Genetic techniques should be useful in this search. Amongst mutants with altered sensitivity to anesthetics should be some that are due to alterations in the amount or structure of the putative anesthetic target. Such mutations might be distinguished by their common effect on a variety of anesthetics, suggesting that they affect some final common pathway involved in consciousness. We have explored the anesthetic Halothane (CF_3-CHCl Br) as a possible agent for selection of such mutants. To begin, we have established the dose-response curve for Halothane anesthesia. Remarkably, this curve is qualitatively and quantitatively similar to that seen for vertebrates. A search is underway for anesthetic-resistant and-sensitive mutants.

To provide the substrate for this search we have initiated a program of chemical mutagenesis of Drosophila. We have established large laboratory populations of suitably marked male and female flies to facilitate detection of recessive mutants. In addition, we have investigated protocols for administering mutagens to male flies in order to generate a large population of mutated sperm. Our first trials have been only slightly encouraging. Although we were able to demonstrate clear induction of lethal mutations, the incidence of easily scored morphological mutants was low. We are exploring recommended improvements in these protocols.

SECTION ON BIOPHYSICAL CHEMISTRY

David M. Neville, Jr., M.D., Chief

Protein Translocation Across Membranes

Most macromolecular components entering (or leaving) cells are routed through vesicles and never cross the insulating membrane barrier. Certain molecules however do make this transition and in a highly specific manner. Examples are transmembrane proteins which can function as devices to transmit signals across the surface membrane or transmembrane protein channels which transport and thereby regulate flow of ions, electric charges and neutral molecules. The insertion or retrieval of the transmembrane proteins from the plasma membrane to intracellular membrane stores profoundly affects the properties of cells. Yet little is known about the chemical nature of these events. Plant and bacterial protein toxins are another group of proteins which are translocated across membranes and in some cases form ion conducting channels. These proteins report their presence in the cytosol compartment by their toxic enzymatic activities or channel forming properties. Because of this the insertion and translocation process can be monitored, and the protein toxins serve as useful models in the study of membrane translocation events.

The study of toxin transport processes and toxin substrates asks several profound questions.

1) How does a highly charged hydrophilic macromolecule cross the lipid bilayer?

2) What controls the high degree of cellular specificity exhibited by these translocation processes?

3) Once translocation is initiated can the process be reversed?

Over the past three years Dr. Thomas Hudson in our Section has performed studies which shed considerable light on how diphtheria toxin crosses the membrane. Dr. Hudson's work suggests that diphtheria toxin gains entrance to the cytosol compartment by destabilizing the vesicle (or a derivative of this vesicle) into which the toxin is packed. An array of toxin molecules of sufficient size to rapidly inactivate all of the cell's elongation factor II (necessary for protein synthesis) enters the cell in a concerted process. Intoxicated cells are found to consist of two populations, those synthesizing protein and those not synthesizing protein. Over the time course of intoxication the population not synthesizing protein increases. The probability that a bolus of toxin will be released into a cell depends on receptor occupancy. This view of diphtheria toxin entry is radically different from previous models. The biochemical mechanisms responsible for this kinetic picture are providing new insights for the construction of efficient immunotoxins utilizing the diphtheria toxin entry function.

The Design of Immunotoxins for the Treatment of Autoimmune Diseases

It is generally believed that multiple sclerosis, rheumatoid arthritis and early onset diabetes are autoimmune diseases. Increasingly, attention is being focused on provocative reports which indicate that certain subsets of mental illnesses may also have an autoimmune component to their etiology. Autoimmune diseases occur when the immune system's defense mechanisms are turned against a person's own body or parts of the body. The initiating causes of these diseases are poorly understood but both environmental factors and genetic factors seem to be involved.

Current therapy for autoimmune diseases aims at inhibiting the part of the immune system causing the damage. Our Section is working to develop very highly selective drugs to inhibit parts of the immune system such as suppressor T cells or helper T cells (T-4 lymphocytes). To do this, we use a totally new class of drug: immunotoxins. Immunotoxins consist of a powerful toxin linked to a monoclonal antibody which can seek out and bind to only certain types of cells. The toxins which come from poisonous plants (ricin) or harmful bacteria (diphtheria toxin) are extremely potent and have specific machinery for entering and killing cells.

The goal of our program is to understand the toxin entry machinery sufficiently well so that we can make therapeuticaly useful selective reagents. To this end Dr. Jon Marsh, working in our Section has elucidated the major variables which lead to efficient entry of the toxin portion of immunotoxins into the targeted cell; affinity, receptor number, and of primary importance, an entry function located on or near the toxin B chain receptor binding site. Manipulating this entry function to only operate on targeted cells is the goal of current research.

ANNUAL REPORT OF THE LABORATORY OF NEUROCHEMISTRY
NATIONAL INSTITUTE OF MENTAL HEALTH
OCTOBER 1, 1985 THROUGH SEPTEMBER 30, 1986

Seymour Kaufman, Chief

During the last year, we have continued our basic and clinical studies of the tetrahydrobiopterin-dependent aromatic amino acid hydroxylating systems.

Results of our studies of the limited proteolysis of rat liver phenylalanine hydroxylase have led to the formulation of a general model of the structure of the enzyme. It is composed of a regulatory domain and a catalytic domain. The former is located toward the amino-terminal end of the molecule and extends, at most, about 100 amino acid residues in from the amino terminal end. This region contains the phosphorylation site that is involved in regulation of the enzyme's catalytic activity. The catalytic domain is located toward the carboxy-terminal end of the molecule and is about $M_r = 36,000$. The carboxy-terminal end, however, is not essential for either catalytic or regulatory activity because a fragment of approximately 40 amino acid residues can be clipped from this end without significally affecting the hydroxylase activity. Since our model can help to predict whether an amino acid substitution will affect catalytic activity, it has already proven to be useful in interpreting data from structural studies of the altered hydroxylase that is present in some phenylketonuric (PKU) patients.

Our studies of kidney phenylalanine hydroxylase have demonstrated that the enzyme in this tissue is not only in a super activated state compared to the liver enzyme, but in addition, that its activity is not under the same hormonal control as is the liver enzyme. An understanding of the structural basis of the kidney enzyme's unusual state of activation would probably provide a clue to a novel mode of increasing the activity of phenylalanine hydroxylase. Since low activity of this enzyme is known to be incompatible with normal brain development, a more complete understanding of the factors that activate the enzyme would be of value.

We have found that the activity of liver phenylalanine hydroxylase can be doubled by feeding rats an iron-rich diet. This is the first example of the regulation of the activity of this enzyme by a normal dietary constituent. This finding suggests that the activity of phenylalanine hydroxylase, as well as of tyrosine and tryptophan hydroxylases, may be controlled by physiological and pathological factors, such as bacterial infection and menstruation, that can alter the tissue level of iron.

We have succeeded in purifying from rat brain all of the enzymes involved in the de novo synthesis of tetrahydrobiopterin (BH_4). These enzyme studies have also provided additional strong support for the proposal that BH_4 is synthesized through a series of tetahydropterin, rather than dihydropterin, intermediates. The availability of the separate pure enzymes should facilitate the preparation of antibodies to these enzymes and, ultimately, to the cloning of their genes. The availability of such genetic probes should help in the diagnosis of a variety of neurological diseases in which altered CNS levels of BH_4 have been found.

Results obtained during the last year have important implications for the present and future therapy of hyperphenylalaninemia caused by a deficiency of dihydropteridine reductase (DHPR). With respect to present therapy, we have finally been able to test a recommendation that we made several years ago, namely, that DHPR-deficient patients should be given supplements of a tetrahydrofolate derivative such as 5-formyltetrahydrofolate to elevate their low tissue levels of folate. We have found that patients given such supplements show marked improvement compared to patients treated with neurotransmitter precursors without such supplementation. In a forthcoming article in the Journal of Pediatrics, I have emphasized that treatment of all patients with DHPR deficiency should include supplementation with a form of tetrahydrofolate.

As far as future therapy of this genetic disease is concerned, our successful cloning (in collaboration with scientists at Baylor University) of the gene for DHPR opens up the possibility of gene therapy for this disorder. Since current treatment for DHPR deficiency is much less successful than that for classical phenylketonuria, exploration of the possibility of gene therapy for DHPR deficiency is probably going to be permitted before it is sanctioned for the classical form of the disease.

Annual Report
Laboratory of Neurophysiology
October 1, 1985 to September 30, 1986
Steven P. Wise, Acting Chief

Fiscal year 1986 has been a year of transistion for the Laboratory of Neurophysiology (LNP). With the loss of the Laboratory Chief, the late Dr. Edward V. Evarts, intensive and self-critical reappraisal has focused on the long-term future of the laboratory and, more importantly, its work. It is worth remembering that Ed Evarts set himself the task of exploring one of the most difficult, but most important problems in neurobiology: the neural mechanisms underlying movement and mood, and the use of memory in guiding behavior. He knew that the problems were difficult, the conceptual and technical pitfalls many, but understood the potential of research directed toward the emergent properties of the central nervous sytems and their interaction. Complete understanding of the brain and its control of behavior can never come about from the study of reduced systems. The study of intact, behaving animals, including humans, not just of "preparations", will be necessary to illuminate the most significant problems in the field.

Linked with considerations of the appropriate biological level on which to study the central questions in neurobiology are the challenges of the so-called "animal rights" movement. There is no denying that the political success of animal rights activists has prompted a serious reconsideration of the methods, goals, and value of basic research on animals, especially primates. Some effects have been deleterious: a promulgation of vague or restrictive regulations; distractions from the scientific enterprise; and potential discouragement of young investigators interested in a scientific career involving animal research. On the other hand, the public and official interest in animal research has led to an increased sensitivity on the part of many scientists to the public's perception of their research and to an appreciation that the conduct of animal research must be beyond even the appearance of misconduct. The supporters of science need to be and *can be* assured that research is conducted and animals are treated in accordance with the highest ethical and moral standards. We cannot accept the extreme claims and aims of the animal rightists, but scientists can and should embrace the cause of animal welfare.

In view of these two challenges, reductionism on the one hand and the animal rights philosophy on the other, this year's annual report will discuss the motivation for and the potential benefits of research in the Laboratory of Neurophysiology more explicitly than in the past. However, we must recognize that our research is at a very basic level and that applications may be far away. Further, in accord with the long history of basic research on animals, we cannot be *certain* that the human ailments discussed below will be ameliorated because of our research. Perhaps these disorders will not, but others will. We cannot be sure; there are no guarantees when dealing with the development of new knowledge. We can only be certain that, as in the past, sound and important biological research will lead to improvements in the human condition, improvements that are manifestly needed if society is to continue the advances of the past four centuries.

Organization of the LNP.

The LNP consists mainly of two groups of investigators. One group is essentially neuroanatomical in its approach. The other group bases its work on the techniques of behavioral neurophysiology pioneered by Evarts. Additionally, Ichiji Tasaki's Unit on Neurobiology continues as a subdivision of the LNP. The reader is referred to Tasaki's individual project report for a description of his work.

The Mission of the Laboratory and Relevance to the Research Program of the Institute.

The Laboratory of Neurophysiology studies brain physiology, anatomy, and pharmacology, principally in rats and rhesus monkeys. LNP investigations focus on the localization of function in well-defined brain areas either by monitoring the activity of neurons within those areas or by studying features such as their cell structure, axonal connectivity, regional brain metabolism, and the distribution of endogenous informons* and their receptors. Our objective is to gain knowledge about the structure and function of the brain, especially those structures involved in the control of behavior. The activity of the laboratory is primarily devoted to basic study of the frontal cortex, a site presumed to be involved in higher brain functions such as reason, attention, forethought, and perception, as well as fine motor capabilities. Most of the remainder of our work is devoted to the other major component of the forebrain, the basal ganglia. Since Laboratory of Neurophysiology research is almost wholly devoted to gaining insight into the functions and mechanisms of the frontal cortex and basal ganglia, it is of great interest to the LNP that diseases involving those two brain regions may be responsible for most or all of the symptoms of schizophrenia (D.R. Weinberger, K.F. Berman, and R.F. Zec, 1986, Arch. Gen. Psychiatry, 43: 114). If we are to understand brain diseases such as schizophrenia, it is necessary to understand the basic biological processes at work in normal brains, possibly including the mechanisms through which the frontal cortex and basal ganglia interact. In addition to schizophrenia, several other neuropsychiatric disorders are known to result from disease of or damage to the frontal cortex and basal ganglia, including but not restricted to: Alzheimer's Disease, which is now believed that a failure of basic cortical mechanisms; Parkinson's disease, which is usually thought of in relation to purely motor disorders, but is now known to have important affective and cognitive symptoms; Huntington's disease, which is also often classed as a motor disorder, but in which memory deficits are the first to appear; and apraxia, the inability to perform skilled motor acts.

About half of the research done in the LNP is performed on rodents, the other half on nonhuman primates. It is especially important to extend the work on rodents to primates since comparison of the telencephalic organization in human forebrains. This fact need not surprise us: rodents and primates (including humans) have been evolving separately and diverging in their brain organization for over 65 million years, monkeys and humans for scarcely half that amount of time. Accordingly, it is much easier and more reliable to infer homologies between monkey and human frontal cortex than between those brain regions monkeys and humans reveals much more similarity than comparison of rodent and in rodents and humans. The same can be said of the basal ganglia. As an example, there is considerable controversy over whether rodents have a prefrontal cortex: Although some cortical areas in rats are called "prefrontal cortex" there is very little morphological or functional evidence for this appellation. Thus, the study of the prefrontal cortex may be impossible in rodents, it is clearly possible in monkeys. As noted in the sections on "Significance" and "Proposed Course" of Wise's individual project report, the Laboratory of Neurophysiology has commenced a long-term study devoted to testing hypotheses about prefrontal cortical organization in primates and its relationship to and interaction with the basal ganglia.

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* an informon is any molecule that transfers information between cells.

Despite the clear relevance of LNP research to human health concerns, we must address the question of utility. If we fail to ask -- and to ask on a regular basis -- if the effort is worth the expense, in terms of both money and animals, then we fail as both scientists and citizens.

As for the animal question, our position is that since an accurate understanding of nonhuman animals inevitably leads to an improvement of the human condition, it is immoral to interfere with legitimate efforts to gain knowledge about nonhuman animals. Others disagree. They are sometimes known as animal rightists, although we prefer the term "anti-research extremists." In the final analysis, we differ with these people in our values. We prefer to contribute to a basic understanding of the brain to the betterment of future human lives. In doing this we impose our values on animals and study them always in an ethiical and responsible manner. Nonhuman animals are accepted to be entities of moral concern, but have intrinsic value or rights only to the extent assigned to them by people. Our adversaries in the animal rights movement think that animals have or should be given the same rights as people and selfishly prefer to impose their values on others who will, in the future, be deprived of the benefits of research in animals. If they are successful it will be the people who suffer most.

As for the financial question, it is worth noting that in the U.S. less than ten cents per person is spent on schizophrenia research each year, less than one dollar per person on all mental health research. But despite the staggering cost of brain disease in terms of both money (over 100 times the research expeditures per year) and inconceivable human suffering, research interest in schzophrenia and certain other brain diseases is on the decline. According to the New York Times (March 16, 1986, page 1), the reasons for this decline include the "lack of any comprehensive theory of the disease to guide research." The work of the Laboratory of Neurophysiology on basic mechanisms and functions of specific brain regions is aimed at contributing to the development of such a theory.

LNP Animal Care and Use Procedures for Nonhuman Primates.

We defer to no one in our commitment to the humane care and study of animals. Our research depends on the health and psychological well-being of our animals, as well as their active co-operation. Cruelty to our animals would not only be morally reprehensible, but also self-defeating. While the following summary of our procedures concerns primate research, work on rodents is conducted in accord with similarly stringent guidelines.

The LNP scientific staff consists mainly of neurobiologists with Ph.D. degrees in one of the biological disciplines. Each of these staff scientists has, before coming to the laboratory, had substantial experience in the handling and care of experimental animals. In addition there are medical staff fellows, with M.D. degrees, who have had extensive clinical experience before coming to the laboratory. All handling and care of the animals is conducted by the scientific staff and one highly experienced member of the technical staff. No students or other inexperienced investigators work directly with nonhuman animals. It is LNP policy that every animal user participates in an NIH-sponsored Investigator Training Course for the Care and Use of Experimental Animals, familiarizes himself or herself with both the NIH Guidelines for the Care and Use of Animals (1985) and the Guidelines for Ethical Conduct in the Care and Use of Animals (1985) of the American Psychological Association, and is enrolled in the Animal Exposure Surveillance Program administered by the NIH Occupational Medical Service. All experiments and procedures have been submitted to and approved by the NIMH Animal Care and Use Committee.

Our procedures for primate research are as follows: rhesus monkeys are obtained in strict accordance with local, state, and federal laws and regulations. These animals are housed in cages that meet or exceed NIH Guidelines. When an animal is selected for participation in an experiment a loosely fitting collar is placed around the monkey's neck. A chain leash can then be attached to the collar for handling the monkey. The monkeys commonly appear apprehensive at first, but they quickly learn that they will not be hurt by handling and become co-operative. When the monkeys become accustomed to handling, they are placed in a primate chair for no more than four hours per day --- typically two to three hours. The monkeys are then operantly conditioned to perform some behavioral task. Operant conditioning is a training procedure in which animals work for rewards; there is no pain or any other aversive stimuli presented to induce the animals to perform the task. Instead the monkeys perform the tasks eagerly in order to receive a sweet juice reward. The monkeys can obtain all of their fluid needs in this way and are allowed to work for all the fluid that they want. In addition to this procedure, every precaution is taken to ensure that the monkey remains in excellent condition for the duration of the study -- typically four to nine months. Their weight is checked several times weekly. If an animal begins to lose weight, its diet is supplemented with fresh fruit and juice as needed to maintain weight. It is common for our monkeys to gain weight during their participation in a research project. When the monkey has learned the task, surgery is performed in order to place a steel "recording chamber" on the animal's head. Each monkey in our laboratory undergoes major surgery once in his or her life, and this is done while the animal is under deep anesthesia. After surgery the monkey is given a strong narcotic to eliminate any pain that might follow surgery. Steel bolts are also placed in the skull for restraining the head during recording sessions. Acrylic cement is then placed around the chamber and bolts, giving the appearance of a 'headdress'. The recording chamber and bolts causes no pain for the monkey and are carefully monitored by experienced personnel for inflammation or infection. We have never had a case of a serious infection with our procedures. When the monkey recovers completely from the surgical procedure, three to seven days later, the recording sessions begin. Typically, the monkey eagerly begins the daily recording sessions by voluntarily jumping into the chair and co-operating with the investigator in fastening the head restraint. It is essential to reiterate that the health of the monkey is an essential feature of the research; if the monkey is uncooperative for any reason, there is no research. At the end of the series of recording sessions the monkey is very deeply anesthetized and sacrificed in a controlled manner. This procedure is necessary for the scientific validity of the research, and attempts to rehabilitate the animal have been judged to be inhumane. We use the minimum possible number of animals, averaging six per year in a laboratory that has six researchers who work primarily with nonhuman primates.

Research Progress Overview.

This section relates the most exciting developments of the past year, in the opinion of the Acting Chief. The reader is referred to the individual project reports for a comprehensive view of research progress in the past year.

Physiology

There are two active neurophysiological projects. The first consists of three parts under the direction of Steven Wise: one conducted by Kiyoshi Kurata, another by Melvyn Heyes, and a third by Eilon Vaadia. The work of Kurata and Wise is directed toward the nonprimary motor cortex and the concept of behavioral set (see Wise's individual project report, Z01 MH 01092-08). The nonprimary motor cortex is one of three major parts of the frontal lobe, the others being the primary motor cortex (studied more intensively in the LNP in years past) and the prefrontal cortex (to be a major focus in the near future). The nonprimary motor cortex is itself composed of at least two functionally distant subdivisions: the supplementary motor cortex (SM) and the premotor cortex (PM) (S.P. Wise, 1985, Ann. Rev. Neurosci. 8: 1).

Two central hypotheses concerning nonprimary motor cortex function were tested in awake, behaving animals this year by Kurata and Wise. Data collection for these successful studies was completed in April and analysis has continued throughout the year. The focus of our studies has been "set-related" activity in PM and, to a lesser extent, in SM. These neurons show markedly increased or decreased discharge when a monkey is withholding movement, previously instructed through a variety of means. The mode of instruction is, thus, the independent variable in our studies, and the magnitude of set-related activity is the dependent variable. Our studies indicate that set-related neurons in the premotor cortex are as active when movements are instructed by arbitrary visual stimuli as when the instruction signals themselves were located near the target of a limb movement. Thus we find support for the hypothesis that PM plays a role in preparing for an action on the basis of abstract external signals. We could not, however, gain support for the attractive proposal (P.E. Roland et al., 1980, J. Neurophysiol. 43: 118; J.C. Eccles, 1982, Arch. Psychiatr. Nervenkr. 231: 423; G. Goldberg, 1985, Behav. Brain Sci. 8: 567) that SM is specialized for control of internally-instructed (as opposed to externally- or sensorially-instructed) behavior. Instead, it appears on the basis of our studies that both PM and SM play a role in those behaviors directed by exclusively internal information processing as well as those directed, in part, by external signals. PM and SM appear to guide behavior whenever a new movement needs to be selected in accordance with rapidly changing, albeit partially predictable, environmental or internal events.

The second part of Wise's physiological project is one that is just beginning. Melvyn Heyes has established a procedure room dedicated to examining cortical interactions with the basal ganglia. Several technical hurdles were overcome in the first part of the fiscal year and data are now being accumulated. While it is too early to evaluate the results, it has been possible for the first time in an awake, behaving animal to identify the cells in the frontal cortex that send their axons to the striatum. By the end of the fiscal year this pilot study should be completed. It is worth noting that Dr. Heyes undertook this important project with the full knowledge that it could fail and that there were many available avenues for "racking up" a long list of publications. It is a testimony to his adherence to the highest scientific principles and practices that he chose this more difficult course over one of the many routes safer for his long-term career goals.

The third part of Wise's project is directly related to the prefrontal cortex. Eilon Vaadia arrived in the laboratory in mid-August and has begun a research project described, below, in the "Proposed Course" section.

The second active physiological project, conducted by Andrew Mitz, involves mapping the supplementary motor cortex and examining the spatial control of movement. Mitz completed a detailed electrical stimulation map of one nonprimary motor cortical field, SM. By applying a modified technique for microstimulation, developed during Mitz's graduate studies, Mitz and Wise were able to demonstrate conclusively: (a) that the SM is topographically organized, (thereby helping to resolve a controversy); (b) that the forelimb area of SM projects to the forelimb area of the primary motor cortex; and (c) that SM contains a representation of eye movements. Detailed cell measurements in SM confirmed the qualitatively observed relationship of the electroanatomically defined SM with the underlying cytoarchitectonics. Mitz will next extend this work to other parts of the frontal cortex in collaboration with the recently-arrived Moshe Godschalk.

Two additional projects are now inactive. The first, of which Von Jennings was principle investigator, was brought to a close in December, 1985. In this exciting project Jennings documented motor cortex signals reflecting errors between intended movements (those that would achieve the animals' goal) and the actual movement that occurs. The actual movement may differ from the goal either because of "random" physiological variation or external interference with the movement. In either case there are neurons in the primary motor cortex that show a faithful reflection of the "mismatch" between actual and intended movements. The concept of mismatch detection may be one which eventually aids in developing a unified hypothesis of frontal lobe function. The second project finished this past fiscal year was started by Evarts and completed by Seth Pullman and Ray Watts. They gained the first evidence in the motor cortex that cells in different layers have different sensitivities to peripheral and cerebellar inputs. They completed work in June 1986.

Anatomy

There are two active LNP neuroanatomical projects. One is conducted by Miles Herkenham, Linda Brady and Stafford McLean. The second is conducted independently by Charles (Chip) Gerfen. Although most of these studies have been conducted on rodents, they are readily understood within the framework of the LNP physiological studies of nonhuman primates.

Herkenham and his colleagues showed that two doctrines concerning neural receptor distribution were erroneous. The first is the expectation that putative neurotransmitters (and other informons) should always be localized near their receptors. Through meticulous analysis McLean and Herkenham showed that this is not at all the case. The exceptions are not rare and this "mismatch" of neuroactive compounds and their receptors presents an important challenge in understanding the physiological significance of these receptor-ligand interactions in the brain. In retrospect such "mismatches" were only to be expected on the basis of long-standing knowledge about the mechanisms of steroid hormone effects and the physiology of hormone receptors generally. However, the specific documentation of such mismatches in the brain has generated substantial interest among investigators studying receptors and promises to be of significance in understanding the "neuroendocrine-like" aspects of brain physiology. Furthermore, these findings are highly relevant to the study of dopamine physiology in the basal ganglia and frontal cortex (see "Proposed Course") since it now appears likely that a nigrostriatal informon, dopamine, functions in a "neuroendocrine-like", "parasynaptic" manner rather than as a "neuromuscular junction-like" synaptic transmitter. The second doctrine undermined by Herkenham and his group is what might be called the myth of "the rodent". Their careful examination of opiate receptor distribution in the hippocampal formation of four rodent species showed an astounding diversity. This diversity indicates that receptor patterns reflect brain adaptations of individual species or higher taxa. Thus, their findings demonstrate the need to study a variety of mammalian species: rats cannot serve as a ubiquitous substitute for humans or other primates; they do not even necessarily serve as a representative rodent. Additional work in collaboration with Richard Rothman and Kenner Rice has been directed to further elaboration of facts about opiate-receptor subtypes and their distribution in the brain. This work, ultimately, will be of relevance in understanding the functions of frontal cortex, which contains among the highest levels of opiate receptors in the rhesus monkey cerebral cortex (S.P. Wise and M. Herkenham, 1982, Science 218: 387).

Brady and Herkenham have been developing techniques for in vivo autoradiographic demonstration of receptor-ligand interactions. If successful, this technique promises to reveal regional changes in the quantity of putative informons released during different states or activities. In vivo autoradiography involves the injection of radiolabeled ligands into the blood or the cerebrospinal fluid (CSF). The distribution of the ligand is then an inverse measure of naturally occurring release of compounds that compete for the same receptor. Brady and Herkenham have established this technique in the laboratory during the past year and are able to compare such in vivo ligand distribution to that observed with in vitro autoradiography. These studies, then, can be viewed as an attempt to link static, anatomical studies of neural receptors to their dynamic changes: in short, their physiology. If these techniques are applicable to primates they will serve as a powerful additional tool for analyzing state-dependent changes in neurochemically characterized systems, such as the dopaminergic nigrostriatal system (see "Proposed Course").

Gerfen, in the second neuroanatomical project, has been working directly on the organization of the basal ganglia. His analysis of dopaminergic and non-dopaminergic inputs to the striatum is now being completed and has revealed that dopaminergic projections arise from a complex "mesostriatal system". The dopaminergic mesostriatal system includes the substantia nigra pars compacta

(SNc), part of the substantia nigra pars reticulata (SNr), the ventral tegmental area (VTA, the main source of the "mesocortical" dopamine projection) and the retrorubal area (RR). All of these subdivisions of the mesostriatal system project to the opiate receptor-poor "matrix" compartment of the striatum, but only the SNc projects to the striatal "patches", which are dense in opiate receptors. Additionally, the SNc projection to "patch" and "matrix" in itself represents separate subsystems. This later point has emerged from Gerfen's developmental studies of the nigrostriatal afferents. The first dopaminergic nigrostriatal afferents to arrive in the striatum terminate in opiate-rich patches. Striatal injection of 6-hydroxydopamine (6-OHDA) at a young age will selectively eliminate this subsystem and leave the SNc projection to the matrix intact. The possible behavioral changes caused by this manipulation are certain to be of great interest and have many important implications in the study of basal ganglia development.

Gerfen's additional studies of compartmentalized input to the striatum have revealed a non-dopaminergic projection from the SNc, one that contains a certain calcium binding protein and projects to the matrix. Other inputs to the matrix compartment also contain this protein. While the functional significance of these calcium-binding proteins remains unknown, it has enabled an improved understanding of striatal compartmentalization in primate brains. Gerfen has shown similar patchiness of a calcium binding protein in the striatum of monkeys and rats. Thus, the dopamine system from the midbrain to the striatum is a far-from-uniform system and the primate mesostriatal dopaminergic system has at least equal complexity to that of rats. These findings open possibilities for dissecting the various functions of basal ganglia subsystems, perhaps including the affective and motor components that are so closely interrelated in the striatum.

Proposed Course.

The behavioral neurophysiology program of the LNP has several specific projects planned or in progress: (1) Functional studies of prefrontal cortex; (2) An analysis of interrelation between frontal cortex and the basal ganglia, especially focused on the dopaminergic system; (3) Mapping studies of motor cortex, including further explorations of its role in goal achievement; and possibly, (4) An examination PM's role in retrieving movements from memory on the basis of arbitrary visual cues.

1) Prefrontal Cortex. We intend to test several hypotheses about the functional organization of the prefrontal cortex in an effort contribute to the development of a coherent theory of frontal lobe function --- one that is valid throughout the primate order and considers the frontal cortex as a whole, including primary motor cortex, supplementary motor cortex, premotor cortex, orbital prefrontal cortex, medial prefrontal cortex, and the dorsolateral prefrontal cortex. To test those hypotheses we will employ the method of recording the activity of single neurons in the cortex of awake, behaving monkeys. The prefrontal cortex project was not originally inspired by, but is nonetheless relevant to the exciting recent findings by Weinberger and Berman and their colleagues at St. Elizabeth's Hospital. They reported that schizophrenics have an inability to increase blood flow in the dorsolateral prefrontal cortex during the performance of a task that they perform poorly. They suggested that the so-called negative or defect symptoms in schzophrenia arise from deficiencies in the dopamine projection to the dorsolateral prefrontal cortex and related

the schizophrenic's deficiency in performance of the tasks to an inability to change and form "central sets" a concept we have discussed at length in our monograph Neurophysiological Approaches to Higher Brain Functions (E.V. Evarts, Y. Shinoda, and S.P. Wise, 1984, Wiley, 198 pp.). Developing the conceptual relationship between behavioral set, as seen at the level of single neurons, and "set", as seen in the performance of a task in which schizophrenics and patients with frontal lobe damage are deficient, leads to several testable hypotheses. We intend to test the hypotheses that: (a) The premotor and/or supplementary motor cortex, but not the prefrontal areas, function in one aspect of behavioral set, the retrieval from memory specific limb movement programs on the basis of a given set of internal and external conditions (cues); (b) The dorsolateral prefrontal cortex functions in another aspect of set, retrieval of expected stimuli from memory and comparing them against incoming stimuli, testing the "hypothesis" that a given stimulus will occur; (c) The orbital prefrontal cortex functions in the determination of whether a given action is successful (i.e., whether the set is the correct one), and then breaks old and forms new sets if it is not; and (d) The primary motor cortex, but no other part of the frontal lobe, compares actual movements to behavioral goals (or motor set) in order to bias muscle activity toward goal acquisition, and does this by sending compensatory signals to either prime mover muscles or other muscles that can assist in goal achievement. It is a corollary to our hypothesis that the prefrontal cortex can be seen to be part of the motor system in the broadest sense, a view supported by current neuroanatomical knowledge, most particularly the finding that the output of the basal ganglia, broadly defined to include ventral striatum and ventral pallidum, is directed to virtually the whole frontal cortex (see Wise's individual project report, Z01 MH 01092-08 LNP, "Major Findings", part 4).

2) Cortical interactions with the basal ganglia. The LNP will begin a series of experiments aimed at an analyses of corticostriatal interactions. There are essentially three aspects of these studies: (a) Identification (by antidromic activation) and study of cortical neurons that project directly to the striatum, first in primary motor cortex, but (with success) ultimately in the prefrontal cortex; (b) Evaluation of the effect of 6-hydroxydopamine (6-OHDA) lesions of the prefrontal cortex on dopamine release and metabolic turnover in the striatum and on the activity of neurons in the dopaminergic substantia nigra or ventral tegmental area; and (c) A study of the activity of dopaminergic nigral cells during different states of behavioral exertion (no movement, steady force production, and ocillating force production) and after various manipulations of the dorsolateral prefrontal cortex (6-OHDA and MPP^+ lesions, lidocaine, reversible cooling and muscimol inactivations, and perhaps mechanical ablation).

3) Electrical mapping of the frontal cortex. Much work remains to be done in understanding the functional organization of the frontal cortex, as a whole, especially its functional subdivisions. Mitz has modified existing stimulation techniques to become much more effective at activating the skeletal and ocular musculature. He will pursue this approach through a systematic mapping study of the entire nonprimary motor cortex and some of the prefrontal cortex, as well. Kurata will, in his final year with our group, study related questions concerning the topographic organization of one nonprimary motor field, the premotor cortex.

4) Role of premotor cortex in motor learning. If time and resources permit, we will test the hypothesis that the premotor cortex acts in the retrieval from memory of a movement on the basis of an arbitrary visual cue (see U. Halsband and R.E. Passingham, 1985, *Behav. Brain Res.* 18: 269). If this is the case, then a sample of premotor cortex neurons before an association is learned should not show any task-related activity, while a later sample should show the now well-known patterns of neuronal activity that accompany the preparation for movement.

Summary of proposed course. We expect to remain focused on the functional organization of the frontal lobe and its interaction with the basal ganglia for the foreseeable future. The neuroanatomy group will, no doubt, continue to make progress in establishing new methods for studying brain structure and development. Gerfen is exploring the mechanisms that determine the patch-matrix organization of rodent striatum during development and Brady, with Herkenham, is exploring new methods for studying the release of informons under physiological conditions. Among the possible future developments we envision for physiological investigation are (1) telemetry monitored studies of striatum and frontal cortex during ethologically meaningful behavior, including social interaction; (2) an examination of prefrontal corticohypothalamic interactions; (3) frontal cortical activity in relation to species-specific stimuli, such as the colors that monkeys prefer on their infant's coats or faces; and (4) comparative neuroanatomical studies of the frontal cortex and its connectional organization. In sum, this plan can be seen as an evolving examination of a broad variety of hypotheses concerning frontal cortex function in an increasingly more ethologically meaningful context.

A Comment Concerning LNP Reorganization.

Since this is the final annual report that the Laboratory of Neurophysiology will file from its ancestral home at the NIH "Bethesda campus", it is appropriate to reflect on its past accomplishments. Obviously, a laboratory is not a building or a corridor or a set of rooms with gas, air, vacuum, and water on tap, but rather is a set of ideas guided by a certain philosophy of science and an effort to achieve a related set of goals. The Laboratory of Neurophysiology throughout its long history, first under the direction of Wade Marshall and the under Evarts has been dedicated to the study of the brain's emergent properties. Its lifetime has witnessed a revolution in brain science of a magnitude that could scarcely be imagined when it was founded in the early 1950's. And the LNP's contribution to that revolution was by no means insignificant. Its "alumni" include such luminaries as Eric Kandel, whose work on the molecular basis of memory has revolutionized that field (there is little doubt that he will be awarded a Nobel Prize for his accomplishments); Emilio Bizzi, whose controversial work on motor control has shaken up a moribund field; Mahlon DeLong, who is the leader in the study of basal ganglia physiology; and Paul MacLean and John Lilly, whose writing have generated popular support for neuroscience, so sorely needed in an era of financial cut-backs and resurgent antivivisectionism.

As some of the current investigators in the LNP join other laboratories within the institute, it is worth noting this event is commonplace in the history of the IRP. A brief perusal of the NIMH organization in 1968 reveals that many laboratories that existed then are gone, many of the current laboratories were but small sections then. Times change and intramural laboratories split and combine with a facility that constitutes a great strength; there is nothing to be regretted in that. The reorganization and relocation of the LNP to the NIH Animal Center is both a continuation and a beginning, one which we approach with optimism.

Annual Report of the Research Services Branch

National Institute of Mental Health

National Institute of Neurological and Communicative Disorders & Stroke

October 1, 1985- September 30, 1986

The Research Services Branch (RSB) provides broad technical support for the Intramural Research Programs of NIMH and NINCDS through (1) research and development in advanced biomedical instrumentation techniques and systems; (2) evaluation, specification and management of computer systems; (3) direction of a program of laboratory animal medicine and care (NIMH only); and (4) provision of other technical services in support of the research program.

The Branch is comprised of three sections:

1. Section on Instrumentation and Computers
2. Section on Laboratory Animal Medicine and Care
3. Section on Research Support (St. Elizabeth's Hospital)

SECTION ON INSTRUMENTATION AND COMPUTERS

The Section on Instrumentation and Computers (ICS) provides technical support for investigators by (1) assessing the instrumentation and computer needs of the investigator; (2) designing, developing and constructing special-purpose electronic and mechanical instrumentation and systems not commercially available; and (3) designing, specifying and managing laboratory computer systems for data acquisition and processing.

Additional services provided by the Section include consultation on measurement techniques, signal processing, noise and electro-magnetic interference in data measurement systems, and equipment purchases. Several formal and informal courses for investigators are taught by ICS personnel; topics include electrical circuit theory, operational amplifier applications, digital logic design, and computer applications.

Due to manpower limitations and economic considerations, the Section is unable to provide the following services: repair of commercial instruments, duplication of off-the-shelf commercially available equipment, and fabrication of non-instrument items (shelves, bookcases, etc.).

When an investigator requires the services of the Section, he first meets with the Section Chief and other personnel as needed to discuss his requirements. On the basis of this meeting, a decision is made as to whether ICS will take on the project. If a commercially produced instrument will satisfy the investigator's requirements, he is advised to purchase it. If custom instrumentation is needed, ICS will accept the project unless we lack the appropriate expertise, or our current work backlog is excessive. In these cases the project may be contracted to a private firm, or the investigator may be directed to the Biomedical Engineering and Instrumentation Branch (BEIB).

When the Section Chief or the Assistant to the Branch Chief agrees to accept a project, the investigator submits a standard work request form (available from ICS), signed by his Lab Chief. This form will state the nature of the instrument or service requested, and should contain as many details and specifications as the investigator can provide.

The project is then assigned to an engineer or computer staff member, who will confer with the investigator to formulate a set of specifications and a timetable and cost estimate for the project. ICS does not charge for services, but the investigator will be billed for the cost of the components used. Upon completionof the project, a memo is sent to the investigator listing the component costs and asking permission to have the Administrative Officer transfer funds from his CAN to the Section's CAN.

INSTRUMENTATION

The Section has a staff of five engineers and four technicians to design, develop, and fabricate electronic and mechanical instruments. The major effort is in the production of electronic instruments for basic neurophysiological research, and for clinical studies involving affective disorders. The following are brief descriptions of representative projects, chosen from a total of 278 projects undertaken this year.

(1) Patient Activity Monitoring System. The Section has continued to develop the Patient Activity Monitor (PAM) and the support hardware and software which forms the system.

(a) Monitor. The current version of the PAM has a memory capacity of 1024 locations and is in its fourth year of production. Fabrication, testing, and calibration of a set of 30 units begun last year has been completed and another set of 50 monitors is now in the initial phase of fabrication. Most of the older versions of the PAM have been retired. Approximately 100 monitors are in use, with the Section providing battery changes and repairs as needed. The injection-molded plastic case developed for the monitor is now being produced with an ABS plastic that contains minute stainless steel fibers. The integral mesh of these fibers appears to provide sufficient electrical shielding to prevent interference from static electricity. A nickel-based conductive paint had been used to solve the static problem but required a separate painting step and caused skin irritation in some patients.

(b) Computer Support. A new PAM computer interface, which uses the RS-232-C serial data format, has been developed. Based on a CMOS 8-bit microprocessor, this interface allows an inexpensive personal computer with a serial port to serve as a readout device. ICS is developing a PAM program for the Apple Macintosh personal computer to support this readout method. This program already provides most of the capabilities of at least 15 PDP-11 programs and is easier to use, maintain, and update. The current version of this program is being tested with the new interface in four laboratories. Four additional interfaces have been fabricated in support of collaborative efforts with groups outside the IRP.

(2) Microprocessor-based Rotometer. A third generation animal rotation monitor was completed that uses an 8-bit microprocessor to determine the clockwise and counter-clockwise rotations of one to four rodents in cylindrical cages and to hold this data for input into the serial port of a Macintosh computer. Software has been written which uses the capabilities of the Macintosh to store the data on disk, and to display the data in real-time histogram form. Powerful statistical programs now available allow complete data analysis directly on the Macintosh. Using this same design approach, a 16-channel rotometer is currently being developed. The combination of the Macintosh and the high-speed microprocessor system reduces the number of integrated circuits in the design by a factor of 20 over previous ICS 16-channel designs.

(3) EMG Data Acquisition System. A three-unit data acquisition system has been completed for use with ongoing EMG studies. The first unit conditions eight analog signals from mechanical transducers (e.g., torque motor position outputs). Each channel consists of an instrumentation amplifier, gain control, switch selectable filter control and DC offset adjustment. The second unit processes eight EMG signals by providing full-wave rectification, low-pass filtering, and gain and offset adjustments. The third unit interfaces the other two to a PDP-11 computer by providing16 channels of buffers and sample/hold amplifiers. Additionally, I/O signals from the computer interface boards are brought out on the front and rear panels for use when needed. Extra computer I/O connectors were added and card slots left open to accomodate future expansion.

(4) EMG/EEG Matrix Interface. A distribution and buffering system is being designed that will individually route fifty neurophysiological signals to any one or more of 110 destinations consisting of A/D converters, strip chart recorders, audio amplifiers, log and antilog amplifiers, and special monitors. To accomodate this extremely large switching network, a programmable pin board with all inputs and outputs buffered is being developed in a standard 8 1/2 X 19 inch rack panel with attached card cage. Custom printed circuit cards have been designed to handle the large number of buffers and ribbon cabling will be utilized for input/output and matrix block connections.

(5) Data Acquisition Preamplifier System. A four-channel amplifier system was designed to interface between low-level spectrophotometer analog outputs and an A/D converter connected to a personal computer via a serial port. The gain for each amplifier channel is continuously variable between 0.5-1000. A dual-purpose offset adjustment was provided that simultaneously cancelled both input and output offset errors in the final amplifier stage, thus eliminating several active components. The system was mounted on top of the A/D converter in a closely-matched plastic cabinet.

(6) Photoglottography Detector. Accurate measurement of larynx activity during speech therapy studies using a bifurcated fiber optic bundle attached to a video camera has been limited by the 60 frames/second video recording rate. Fundamental frequencies of the vocal chords can be as high as 400Hz. In addition, it is desirable to monitor the time course of the opening width of the vocal chords. A self-adhering photodiode transducer was developed that measures both the frequency content and the quantity of light passing through the vocal chords by sensing the light leakage that escapes through the skin just below the Adam's Apple. The device is currently undergoing clinical evaluation.

(7) Isolated Linear Amplifer. A low distortion DC-25kHz optically-coupled amplifier has been developed to generate high-voltage isolated stimuli pulses for cell studies. A feedback type optically-coupled amplifier was utilized for the low distortion isolation and a high-voltage, high-slew rate operational amplifer with switchable gain ranges was used for the final stage. Output characteristics were 20 µsec pulses at +/- 20 volts peak into a 5kΩ load.

(8) Constant Current Stimulator. This device presents a bipolar current pulse through concentric electrodes placed on a subject's forearm as part of stimulus threshold studies. Current pulses from 1 to 31mA are controlled in one milliamp steps by a 5-bit digital word from a PDP-11 computer or manually by binary-weighted toggle switches. A trigger pulse from the computer or a pushbutton generates a bi-phasic stimulus of 2msec. duration. The stimulator design employs a center-tapped 100:1 transformer output stage supplied on the primary by a high-current DC supply in order to provide symmetrical pulses up to 31mA into load impedances equal to or less than 50kΩ.

(9) Dual Electrode Stimulator. This device allows two sets of electrodes to be used for both stimulation of rat brain tissue and for recording from the same sites. The two pairs of electrodes are switched from a strip-chart recorder to two constant current stimulators; after stimulation, the electrodes are briefly depolarized and then switched back to the recorder. This system is a dual version of units previously built by ICS and has been updated to include new technologies. The unit is equipped with logic to sample the stimulus current pulse, hold this value and display it on a panel meter. The display feature allows the user to monitor the stimulus pulses during an experiment.

(10) Attention Monitor. A simple microprocessor-based device has been design to present a challenge test to subjects while they are exposed to a two-hour light stimulation during normal sleeping hours. On the average of once a minute, a four-second visual cue is presented. If the subject responds while the cue is on, a "hit" is recorded; no response results in a "miss" and a short alarm is sounded to regain the subject's attention. After the two-hour session ends, the time course of responses is printed so that the subject's attention pattern can be used in the evaluation of the efficacy of the light stimulation.

(11) Pulse Generator System. A multi-channel timing instrument (pulse generator system) is a vital part of many neurophysiological experiments. Instruments used within the IRP that were purchased about 17 years ago are no longer manufactured and have become unreliable. Newer, commercially available units lack the flexibility and convenience of the older devices. Two years ago, ICS designed a five-channel pulse generator system to fill this void. Six of these instruments were fabricated last year and an additional six units were completed this year.

(12) Neurophysiological Data Preprocessor. A microprocessor system was developed to replace the custom logic circuitry presently used by the Laboratory of Neurophysiology Data Acquisition System. This preprocessor records the times of occurrences of 64 different events and eight different pulses. This information is transmitted to the main processor (a PDP-11 minicomputer) through a parallel interface and the information is coded in such a form as to ensure compatibility with existing software that is used for analysis and display of the data. The preprocessor decreases response time to events and pulses and it frees the main processor for experiment control. Last year ICS constructed six units for the LNP/NIMH and two units for the LNLC/NINCDS. A user's manual for this system is now being developed and a search is being conducted for a private manufacturer to produce and market this system.

COMPUTERS

Small computers are ideally suited for laboratory research in neurophysiology and psychology. They are used in the laboratory for on-line, real-time interactions, process control, and data acquisition. Recorded data may be stored, combined with other data, reduced statistically, transferred to larger computers for further analysis, transformed for presentation graphically or mathematically, and the results may be printed or plotted. Increasing use is being made of the small computer for processing the text of scientific papers and communications. Data base management is now available for the small computer, as are limited management information systems.

Techniques have been developed for image processing which are applicable to many diverse experimental systems, ranging from autoradiographs of brain tissue sections to the analysis of two-dimensional electrophoresis gels.

Larger minicomputers, the so-called super-mini's, have been reduced in price and are now available for functions formerly performed by larger time-shared systems. These systems allow applications in modeling, curve fitting and statistical treatment that would be prohibitively expensive on large systems.

Inexpensive personal computers are proving useful for dedicated applications. Many scientists are developing software for these computers, which they offer to the scientific community at low cost. PCs will become increasingly useful in the laboratory and their potential should be exploited.

Microcomputers incorporated in the design of biomedical instrumentation provide a savings in design and fabrication time for instruments, and a more flexible system than one based on discrete components.

The Instrumentation and Computers Section is actively involved in the applications of small computers in the IRP. By integrating the functions of biomedical instrument design and laboratory computer systems with software designed specifically for the research community, the Section offers computer support services for a broad range of scientific disciplines.

LABORATORY COMPUTERS

The design goal for the laboratory instrument computer is to provide maximum function, tailored to the specific experimental design, with minimum cost. ICS provides consultation on the specification and selection of laboratory computers for new applications; conducts systems studies in collaboration with the scientist; and helps the scientist in the procurement, installation and maintenance of the equipment. In support of these efforts, ICS maintains a PDP-11/73 central computer in Bldg. 36 for program development and training. Additionally, a multi-user VAX-11/750 managed by ICS in Bldg. 36 provides high-capacity data storage, and efficient data processing, including graphic functions with plotting and printing on a high-resolution laser printer.

ICS provides training for the scientist or support personnel who will be programming and maintaining the laboratory computer system. Personnel limitations make it difficult for ICS to provide complete programming for specific individual applications, so such programming must be supplied by the laboratory. ICS computer personnel are always available for consultation, training, and help in debugging, as well as assistance in the selection of part-time programmers or consultants. Commercial software packages or applications from other research labs are often available, and ICS will evaluate such systems.

ICS develops and maintains a library of procedures which are written specifically for the laboratory computers used in the intramural community. These procedures are designed to be incorporated into the users' programs. In addition, ICS will aid the investigator in writing the difficult time and data dependent sections of real-time programs. ICS also develops some application programs which will have wide use within one or more laboratories or will support data acquisition hardware developed by ICS.

There are now more than 60 minicomputers in the program; many of these systems have been in use for years. A significant number of library procedures and general-purpose application programs are used on these machines. As experimental protocols develop and change, software changes are often required, so program maintenance is a continual and time-consuming function of the Section. This effort is aided by structured programming techniques and standardization of laboratory computers and peripheral equipment.

VAX COMPUTER SYSTEM

ICS manages a multi-user VAX-11/750 computer system that is available to all IRP investigators. The VAX is located in Building 36, in space furnished by the Laboratory of Cerebral Metabolism, NIMH. Users in the building can have hard-wired cable connections installed for high-speed communication with the VAX, or can access the computer on a dial-up basis. In striving to improve the VAX as a useful research tool, ICS has installed a variety of new software, described below.

To aid research in molecular biology, the Section has assembled a system for convenient entry, analysis, and publication of nucleic acid and protein sequences. This comprehensive set of software and sequence databases provides IRP investigators with a no-fee alternative to a similar system on the DECSystem-10. An important part of the system are Pearson's FASTN and FASTP programs which enable an investigator to quickly determine whether a new sequence is homologous to any entries in the latest GenBank™ nucleic acid database or NBRF protein database that are maintained on the VAX. Also available are Staden's DBSystem for management of large shotgun sequencing projects, Staden's AnalySeq and Diagon programs for analysis of newly-acquired sequences, and NBRF's NAQ, PSQ, ALIGN, and PRPLOT programs.

For graphics support, ICS has developed PlotLib, a device-independent graphics package, which generates graphs on numerous display terminals and hardcopy devices. PlotLib-based software and a high-resolution Talaris laser printer permit publication-quality graphs and documents to be quickly and easily produced with a variety of programs. Printed documents may incorporate superscripts, subscripts, and Greek letters.

DATAPLOT, an interactive program featuring a high-level language for curve fitting and graphics, is now installed on the VAX. Similar to MLAB on DCRT's DECSystem-10 computer, DATAPLOT is a tool for experimenting with mathematical models, as well as summarizing and analyzing data. Publication-quality graphs produced by DATAPLOT can be plotted on the Talaris laser printer.

An Ethernet local area network is being installed on the second floor of Building 36. It will allow experimental data acquired by laboratory PDP-11s to be quickly transferred to the VAX for analysis and plotting. This network can be extended to other floors in Building 36 if the initial installation on the second floor is successful.

IMAGE PROCESSING

ICS maintains a general purpose image processing system consisting of an Optronics rotating drum film scanner, a Gould/DeAnza image array processor, and a PDP-11/34 computer. Images to be processed may be obtained by scanning autoradiographs, x-ray film, or photographic negatives, or by using images generated by CAT or ECAT scanners. A camera station is available to generate color hardcopy using Polaroid SX-70 or 35mm film.

Software packages that are easy to learn and use have been developed to provide an extensive and expandable repertoire of basic image processing functions. Special purpose functions can be developed to meet specific user requirements. The facility is useful for numerous applications involving evaluation and quantification of biomedical images. The two primary applications of the system are the densitometric analysis of autoradiographs of brain or tissue sections and the analysis of two-dimensional electrophoresis gels.

ICS has developed a new PDP-11/73 based image processing system that is capable of using these software packages. This system uses a TV camera for digitizing images instead of the rotating drum film scanner. Unlike the drum scanner, which can only digitize transparencies, the TV digitizer permits any object that can be placed under a camera to be digitized. This system will have a high-speed Ethernet link to the VAX-11/750.

PERSONAL COMPUTERS

ICS has evaluated Apple Macintosh personal computers for potential use in both scientific and administrative applications. The Macintosh was chosen for its ease of learning and use, advanced design, and high quality graphics. It has proven useful in many areas, such as scientific word processing, where its ability to easily produce text containing equations, Greek letters, superscripts, and subscripts make it a very cost-effective alternative to expensive and inflexible dedicated word processors. The Macintosh has also reduced both the cost and time involved in making posters, slides, and publication-quality charts and tables. When used with the Apple Laserwriter printer, print quality is as good as, or better than, that of a dedicated word processor.

The Macintosh has also proven to be an effective graphics workstation for use with the VAX, DECSystem-10, Wylbur, and MEDLINE. An inexpensive program called VersaTerm allows the Macintosh to function as either a VT-100 compatible, full-screen editing terminal or as a Tektronix 4014 compatible graphics display terminal. VersaTerm allows text and graphics generated by the VAX to be printed on any Macintosh printer and it supports file transfer between host computers and Macintoshes.

In order to help IRP scientists with their scientific word processing and graphic workstation tasks, ICS maintains two Macintosh personal computers connected to an Apple LaserWriter printer in the central computing facility in Bldg. 36. ICS selects new Macintosh programs for statistical analysis, plotting, drafting, word processing, etc. and makes them available for evaluation at this facility.

The Macintosh is being used in four ICS projects for low-speed laboratory data acquisition and control. The first project involves presenting stimuli (various words or geometric designs) to Alzheimer's patients with recording of patient responses. A second project uses the Macintosh to control and collect data from an HP 8450 Spectrophotometer. The third project uses the Macintosh to log data generated by a four-channel rodent rotometer developed by ICS. The fourth project uses the Macintosh as a readout station for the Patient Activity Monitors supported by ICS.

While the main emphasis in personal computers by ICS staff has been on the Macintosh system, a smaller effort is under way to develop expertise with the IBM personal computer. One staff member has become involved in the DCRT Personal Workstation Office lead user program and continues to gain expertise through advanced DCRT courses. ICS is developing an IBM-AT PC system as an engineering workstation and as a prototype for laboratory data acquisition projects.

MICROPROCESSORS

High-speed 8- and 16-bit microprocessors have become important design tools for the development of biomedical instrumentation by ICS engineering staff. Current CMOS low-power versions of these chips allow the design of both smaller, more reliable bench instruments and more intelligent portable devices. ICS maintains a complete Intel system for development of projects based on the 8085 and 8088 family of chips. Software development tools running on the Macintosh are available for support of Motorola/Hitachi 6801 and 6805 single-chip processors.

ENGINEERING, COMPUTER, AND FABRICATION SERVICES

This table shows the distribution of the Section's workload among the various laboratories and branches. We have listed only the major users.

LABORATORY OR BRANCH	PERCENT
Medical Neurology, NINCDS	13.68
Neurophysiology, NINCDS	12.79
Clinical Psychobiology, NIMH	11.67
Biological Psychiatry, NIMH	8.57
Cerebral Metabolism, NIMH	4.65
Experimental Therapeutics, NINCDS	4.62
Biophysics, NINCDS	4.24
Child Psychiatry, NIMH	3.46
Clinical Science, NIMH	2.86
Neuropsychology, NIMH	2.62
Clinical Neuroscience, NIMH	2.45
Cell Biology, NIMH	2.45
Neurophysiology, NIMH	2.38
Neurochemistry, NINCDS	2.25
Neurobiology, NINCDS	1.65
Molecular Biology, NIMH	1.43
Neurochemistry, NIMH	1.41
Molecular Genetics, NINCDS	1.16
Neural Control, NINCDS	0.84
Neuropsychiatry, NIMH	0.81
Surgical Neurology, NINCDS	0.74
Central Nervous System Studies, NINCDS	0.53
Experimental Neuropathology, NINCDS	0.51
Psychology & Psychopathology, NIMH	0.34
Neuropathology & Neuroanatomical Sciences, NINCDS	0.31
*NIMH (TOTAL)	45.74
*NINCDS (TOTAL)	43.74
*NICHD (TOTAL) **	8.22
*NIAAA (TOTAL)***	2.30
	100.00

*These figures represent our total effort; they include labs not listed individually.

**This figure represents our support of NICHD which loans the Section one FTE.

***This figure represents our support of collaborative efforts between NIAAA and NIMH.

SECTION ON LABORATORY ANIMAL MEDICINE AND CARE

The Section on Laboratory Animal Medicine and Care provides a comprehensive animal care and use program for the NIMH Intramural Research Program. The average daily animal inventory is 3,500 animals, including mice, rats, hamsters, guinea pigs, rabbits, and nonhuman primates. The Section has responsibility for 25 rooms, comprising approximately 10,000 sq.ft. The staff includes a laboratory animal veterinarian, 15 laboratory animal care technicians, a biologist, and one part-time secretary. Approximately 250 IRP investigators are involved in animal research, and depend on the Section for animal supervision and advice on animal care and use issues. Specific LAMC activities include the following.

Animal programs required by ADAMHA and NIH regulations:

Clinical care of animals. 7 day clinical care is provided for all IRP animals.

Animal use forms. The Section monitors the documentation and approval process for animal usage.

NIH "Guide for the Care and Use of Laboratory Animals". The Section is responsible for assuring IRP compliance with the NIH regulations in the guide.

Animal facility site visits. Formal site visits are conducted, and appropriate documentation of findings is provided.

Animal Research Committee meetings. The Section is responsible for assuring that the Committee meets regularly, and for providing full documentation of the deliberations.

Animal facility accreditation. A plan for AAALAC accreditation of the facilities at NIH, St. Elizabeths, and Poolesville is being developed.

Surgical facilities. The Section provides a fully equipped surgical facility, and assists investigators with anesthesia and surgical techniques.

Experimental rooms. The Section provides and maintains several nonhuman primate experimental rooms.

Clinical records. A system of maintaining clinical records for all experimental animal subjects is being developed.

Employee health system. The Section maintains a system for monitoring the health of employees who have regular contact with animals.

Animal room sanitation. Adequate sanitation of all animal facilities must be provided.

Provide attending veterinary services for St. Elizabeths and Poolesville facilities.

Prepare annual FDA report of animal usage.

Prepare Annual Report of Research Facility as required by ADAMHA.

Provide space for storage of animal care equipment.

Prepare OPRR, NIH assurance statement.

Other programs and activities:

Animal and space inventory system. Define equitable distribution of animal holding space, and monitor usage by maintaining a comprehensive and current inventory of animal holdings.

Animal ordering system. Maintain a centralized system for ordering animals.

Provide a stable source of supply of nonhuman primates.

Provide adequate security for the animal facilities, to guard against unauthorized intrusions.

Provide a Rhesus monkey nursery facility, attended 18 hours/day, every day.

Maintain membership and active involvement in the following committees: NIH Animal Research Committee; Interagency Animal Research Committee; ADAMHA Animal Research Committee; and BID Veterinarians Committee.

SECTION ON RESEARCH SUPPORT (ST. ELIZABETHS HOSPITAL)

The Section on Research Support (SEH) consists of eight individual functions (animal care, computer services, library, instrumentation, graphics, electronics, glassware, photography and receiving) providing support services for the psychiatric and biomedical research laboratories and administrative staff.

The animal care unit provides housing, maintenance and post-surgical care for 3,000 research animals including rats, mice, guinea pigs, rabbits, and nonhuman primates. The facility is staffed daily, providing specialized care and services to investigators as needed. AAALAC accreditation pre-requisites have been met, and full accreditation is expected to be awarded in the coming year.

A Digital Equipment Corp. VAX 11/750 is the primary computer in the William A. White Bldg. computer facility. The system is available 24 hours a day, and supports both the scientific and administrative needs. The present configuration has 8Mb of memory, a 456Mb Winchester type disk, a 256Mb removable disk, a 800/1600 bpi magnetic tape drive, and five 300/1200 baud dial-up lines. In-house communication with the VAX is 9600 baud, accomplished through a Xyplex front end which offloads all I/O from the VAX, allowing access to the system from virtually anywhere in the building. Software includes SAS and SAS GRAPH, Fortran 77, Pascal, DATATRIEVE, VAXADE, CDD, and FMS.

The glassware unit processes an average of 75,000 pieces of apparatus yearly, and the receiving unit provides daily receipt and delivery of equipment and supplies.

The graphics department provides original art in a variety of mediums including pen and ink, carbon dust, oil, acrylic, watercolor, air brush, and computer-generated graphics. Over 1300 original medical illustrations were produced this year for publication in journals.

The instrument shop provides support for the investigative and clinical staff by designing and fabricating specialized equipment and systems not commercially available. The engineering technician was reassigned to the NIH campus where his skills were vitally needed, due to the loss of two technicians there.

The library maintains 17,000 volumes and journals and is the focal point for literary resources for both the NIMH and SEH medical staff. As a Federal MEDLINE center, the library provides online computer literature searches, and has the DIALOG information system available, allowing access to approximately 200 data bases. Through interlibrary loans, materials can be obtained from the National Library of Medicine, the NIH library, and the Library of Congress.

The photography unit provides a variety of services, including color and black and white film development, copy and reproduction work, X-ray to black and white negative conversion, slide duplication, printing, and custom photographic needs as requested. This past year the photography unit produced slides and prints for over 200 publications and presentations.

Report of the Associate Director for Research at Saint Elizabeths Hospital
and Chief of the Neuropsychiatry Branch
Intramural Research Program
National Institute of Mental Health

This year has proven once again to be a fruitful and successful one for all of us at the NIMH Intramural Research Program at Saint Elizabeths Hospital. Congratulations are in order to Dr. Weinberger, who won the Arthur S. Flemming Award from the Jaycees for outstanding scientific achievement in the Federal Government, and to Dr. Berman, who won the A.E. Bennett Award from the Society of Biological Psychiatry. Also Dr. Bridge, received the Geriatric Award. I received the Arthur P. Noyes Award from the State of Pennsylvania for schizophrenia research and the Margaret Byrd Rawson Award. Congratulations go to Dr. Kirch, who has now become our Deputy Clinical Director for the William A. White Division.

We greatly miss Drs. Costa and Guidotti, who left NIMH to take an exceptional position in industry in association with Georgetown University. These scientists were a vital part of the Intramural Program at Saint Elizabeths Hospital.

Sharing some of our other highlights with you, we have published or have in press, approximately 150 papers, enjoyed 17 invited seminar speakers and have had 13 guest researchers working with our regular staff. Over 400 tests, or approximately 133 subjects were studied on our regional cerebral blood flow (rCBF) machine under the direction of Drs. Berman and Weinberger. These subjects included normals, schizophrenics, Alzheimer's patients, Huntington's patients, and patients with Parkinson's disease. The blood flow team also collaborated with other laboratories to analyze patients with Down's syndrome, dyslexia, and obsessive-compulsive disorder.

This year's data brings us to a grand total of over 1500 individual procedures that have been successfully completed in the rCBF laboratory. Data management techniques for the resultant informational base and statistical methods to address the complex interrelationships of regional brain function have been and continue to be developed. During the past reporting year, Drs. Berman and Weinberger used the rCBF machine to investigate cognitive function in patients with Parkinson's disease before and after dopamimetic treatment. The data collected strongly suggest that dysfunction of the prefrontal cortex underlies some of the cognitive symptomatology of Parkinson's disease and that reduced dopamine afferentation may be the pathophysiological mechanism. The blood flow team is now progressing to new protocols for the evaluation of the effects of apomorphine in schizophrenic patients.

Also, the MRI (Magnetic Resonance Imaging) equipment used by our Clinical Neuropsychiatry Section has made it possible for Drs. Weinberger and Cadet to study the neuroanatomical abnormalities of the possible underlying pathology of schizophrenia by a non-invasive, non-radiologic technique. Meanwhile, Dr. Craig Karson is currently examining CEAM (Computerized EEG Activity Mapping) in chronic schizophrenics in a drug-free resting state as well as an active medicated state in order to better ascertain the pathological process involved in schizophrenia. Drs. Jaskiw and Illowsky have continued the investigation of structural pathology in the brains of schizophrenic patients and are using our recently installed CT scanner. In the near future we are hoping to get a SPECT (single photon emission cell counter). We are very thankful for the VAX computer system which has greatly enhanced our productivity as well as the quality and type of research that we are able to do. The system is currently supporting more than 90 users and is printing an average of 16,000 pages a month, saving us approximately $60,000 per year.

Dr. Jean Cadet has continued his study of the toxic effect of various substances on the behavior of rats in an effort to achieve a better understanding of neuropsychiatric movement disorders in humans. Data suggest that persistent dyskinesia may be associated with increased activation of protein kinase C in the central nervous system.

Dr. Terry Goldberg spent his first year at NIMH on several projects. For the purpose of rehabilitation techniques as well as general understanding of the presumed frontal lobe dysfunction in schizophrenics, he developed methods of teaching the Wisconsin Card Sort test to patients and observing which interventions produce improvements in cognitive thought processes. He also developed an auditory analog of the card sort in order to further validate the test and to facilitate cognitive activation in EEG-BEAM studies. The card sort test was studied in relation to other frontal lobe tests to determine their sensitivity and possible hierarchical arrangement.

Dr. Gregory Straw, another newcomer to NIMH in the past year, has been investigating the pathophysiology of retinoic acid action in vivo in rats. Early results have shown statistically significant changes in the blood levels of haloperidol and one of its metabolites after the concurrent administration of 13-cis-retinoic acid to rats. Dr. Straw also investigated the possibility of beneficial effects of calcium channel inhibitors in neuropsychiatric disorders.

Dr. William Freed, Chief of the Preclinical Neurosciences Section, continued his animal studies on the development of pharmacological agents for the treatment of PCP abuse as well as making important progress in brain tissue transplantation. His finding that brain injury has a trophic effect on dopamine-containing neurites is of potential importance for the understanding of the developmental and trophic influences on the dopaminergic system and its possible dysfunction in schizophrenia. Subsequent studies using this paradigm may provide useful information about the effects of brain injury on neuronal circuits.

My own work with brain tissue grafts in the rhesus monkey have progressed well with the development of an instrument which allows for accurate insertion of the graft and which has subsequently increased graft survival.

Dr. Myles Jaffe, another newcomer, concentrated his efforts running electroretinographic assays of schizophrenic patients in order to determine whether the ganglion cell layer of the retina is perturbed by the disease similarly to blue-sensitive cone function in previous studies. Such findings may possibly serve as a model of less accessible CNS structures.

Dr. Anita Feenstra, while continuing a somatostatin project to observe RNA distribution in the brains of schizophrenics and Huntington's disease patients, has also initiated a study of possible retroviral activity in the lymphocytes of patients with schizophrenia.

Also in the realm of molecular biology, Dr. Anne-Marie Duchemin made important discoveries about the gene coding for cholecystokinin in the rat brain. She is still studying the expression of the gene for tyrosine hydroxylase as well as trying to clone the gene which codes for lesion-induced trophic factors in the brain. Dr. Duchemin also began a study that could shed light on the pathogeny of some degenerative brain disorders. By testing the effect of cerebrospinal fluid of schizophrenics on a culture of sympathetic neurons, she discovered that the CSF of some schizophrenics contained a high neurotrophic activity for these neurons.

Dr. Farouk Karoum continues to study the role of catecholamines in mental illnesses, as well as obtain information on the metabolism of ingested L-dopa in rats. This study may prove useful in the future design of of L-dopa treatment in parkinsonism.

While our brain bank is increasing under the direction of Dr. Joel Kleinman, we continue to lend samples to various investigators as well as to employ them for our own use. At least 10 different projects in the IRP, NIMH have used samples from our collection. We collected approximately 100 brains over the past year, 50% of which were suicide cases and 12% of which were schizophrenics. The rest included alcoholics, drug addicts and normals. Dr. Kleinman's present study of post-mortem brain tissue focuses primarily on neurochemical hypothesis in schizophrenia and suicide.

Dr. Llewellyn Bigelow, Associate Clinical Director, modified the Brief Psychiatric Rating Scale to enhance its reliability and devised a training program to implement greater interrater reliability in the clinical setting. He also opened up a new frontier on the understanding of migraine by his work with anionic polyelectrolytes. Also on the clinical side, Dr. Darrell Kirch developed a "Negative Symptom Rating Scale" to more efficiently measure the defect state of schizophrenia.

Dr. Frank Putnam's work on multiple personality disorder has continued to receive recognition by the broader scientific community. The self-administered questionnaire which he developed to measure dissociative phenomena has been adopted by more than 20 research projects around the country and is currently being used by the National Academy of Sciences in a large scale study of post-traumatic stress disorder in Vietnam Veterans.

Dr. de Medinaceli has successfully developed a model for working on spinal cord injuries in rats. A major breakthrough in this process was the discovery of a vein between the multilocular adipose tissue and the left azyygous vein near the fifth thoracic vertebra. This vein, which is a convenient landmark for dissection, had been previously unidentified by the biological community. Preliminary steps toward an orthopedic approach to spinal cord repair will be published soon.

Drs. Jeste and Lohr have made further progress in understanding the neuropathology of aging and various disorders as well as continuing to study the biochemical abnormalities in the brains of patients with tardive dyskinesia. They are now testing the efficacy of antioxidant therapy in treating this disease.

In the realm of synthetic microstructures, Paul Oliver has continued apace with recording neurons growing on a microchip. This project has demonstrated the feasibility of using solid state devices for long-term recording of nerve cells. We also had our first microstructure conference at Arlie House which was attended by about 100 scientists from around the world.

Dr. Janice Stevens has continued the search for a treatment for epilepsy by grafting GABA'ergic brain tissue to affected brain areas in rats. Dr. Stevens is simultaneously exploring post-mortem brain tissue of patients with schizophrenia in order to determine the possibility of a viral etiology of the disorder.

Last, but not least, Dr. Shore is proceeding with his research on national arrest records.

Recently, I had the opportunity to organize a two-day celebration conference for the 40th Anniversary of the Mental Health Act. The celebration was a success and it was good to see many old friends again.

Amidst so much productivity, we are sorry to be losing some of our staff, though we know they will continue to achieve great things for the scientific community at their new positions. Among these are Drs. Putnam, Wang, and Jeste as well as Ms. Mary Iadorala and

Ms. Naomi Driesen from the blood flow group. Drs. Shore, Jaeger, Shelton, Bracha, Kaufmann, Grebb, Costa, and Guidotti are also deeply missed as are Ms. Johnson, Ms. Danzig and Mr. Thompson. We wish them all the best of success.

On the other hand, we would like to welcome Drs. David Daniel, George Christison, Richard Suddath and Richard Coppola, and trust that their time with us will be both fruitful and enjoyable.

Last but not least, I would like to say thank you to some of the administrative staff that keep the laboratory organized and running smoothly. I will certainly miss Dr. Evan DeRenzo and Mrs. Nancy Bryant, both of whom have moved on to areas outside of NIMH. Meanwhile, I welcome Katarina Clark. Once again, I look forward to reporting on all of our progress in next year's annual report.

Annual Report
Laboratory of Preclinical Pharmacology
October 1, 1985 Through September 30, 1986
H.-Y. T. Yang, Ph.D., Acting Chief

The Laboratory of Preclinical Pharmacology has undergone a major change in the last year due to the retirement from the government service of the Laboratory Chief, Dr. Erminio Costa. The laboratory is currently comprised of two groups, Molecular Biology and Receptor Pharmacology headed by Drs. J. P. Schwartz and D.-M. Chuang respectively, and one section, Neuropeptides headed by Dr. H.-Y. T. Yang. The research of the laboratory is focussed on the areas of (1) regulation of opioid peptide synthesis studied by molecular biology approaches, (2) molecular mechanisms of neurotransmitter receptor regulations, (3) mechanism of differentiation of neural crest cells, (4) opioid receptor model, (5) roles of endogenous putative opioid antagonist peptides in pain modulation and (6) functional role of neuropeptide Y in adrenal glands.

The group on Molecular Biology continues to study the regulation of enkephalin biosynthesis and turnover by the method comprised of measurement of precursor mRNA, precursor and peptide. In this research two model systems are used: (1) regulation of synthesis of cotransmitters enkephalin and catecholamines in bovine chromaffin cells and (2) regulation of enkephalin synthesis by opiate receptor in NG-108 cells. Recently, Dr. Schwartz has developed a highly sensitive DNA-mRNA hybridization technique and is planning to study proenkephalin expression in primary cultures of striatal cells and also nerve growth factor mRNA in brain of pcd mutant mouse.

The group on Receptor Pharmacology is focussed on biochemical mechanism of receptor regulation including the β-adrenergic receptor desensitization via receptor internalization and the receptor-mediated phosphoinositide turnover. The β-adrenergic receptor internalization is currently studied immunohistochemically with specific β-receptor antibody. The receptor-mediated phosphoinositide turnover is carried out on NCB-20 cells, a neurohybrid between neuroblastoma and fetal brain cells, rat aorta, brain slices and primary culture of cerebellar granule cells. The emphasis of this study is to find a appropriate model to identify the physiological substrate for the protein kinase C which is activated during the stimulation of the phospholipase C linked receptor. This group is also engaged in the investigation of mechanisms involved in differentiation of neural crest cells using PC12 cells as a model system. The differentiation promotors studied are Na-butyrate and nerve growth factor.

The Neuropeptide Section has continued to focussed on the two newly characterized endogenous putative opioid antagonist peptides, phe-leu-phe-gln-pro-gln-arg-phe-NH_2 (F-8-F-NH_2) and ala-gly-glu-gly-leu-ser-ser-pro-phe-trp-ser-leu-ala-ala-pro-gln-arg-phe-NH_2 (A-18-F-NH_2), and neuropeptide Y (NPY). In order to further elucidate the functional role of the peptides, F-8-F-NH_2 and A-18-F-NH_2, specific antibodies were raised and distribution of these two peptides in CNS was studied by both radioimmunoassays and immunohistochemical techniques. Current studies on these two peptides also include construction of a cDNA library from bovine brain hypothalamus (in cooperation with Dr. S. Sabol at the Lab. of Biochem. Genet., National Heart, Lung and Blood Institute) in an attempt to clone the genes for the two peptides, A-18-F-NH_2 and F-8-F-NH_2. In the project on NPY, the physiological release of NPY was studied by using retrogradedly perfused adrenal gland and the possible role of NPY on the catecholamine secretion was investigated using cultured bovine adrenal chromaffin cells. Recently, we have also initiated a project to purify the NPY-like peptide which was found to appear in the adrenal glands of old rats. The final goal of this project is to characterize this unidentified peptide chemically and also biologically. This section is also engaged in a project on opiate receptor. Using ligand binding, autoradiography, chronic drug treatments, the goal of this study is to develop a reasonable model to explain varied physiological data.

The following summaries of the year's research activities have been prepared by the group leaders and also guest researchers, Dr. Byrd and Dr. Rothman.

Group on Molecular Biology (Head: Dr. J. P. Schwartz, Ph.D.)

The group on Molecular Biology has continued studies on neuropeptide biosynthesis and turnover by utilizing the method we developed, which combines measurements of precursor mRNA, precursor, and peptide (as well as medium content of peptide for cell cultures). Bovine adrenal chromaffin cells provide a model system in which to investigate regulation of the synthesis of co-transmitters, since enkephalin peptides are co-stored with catecholamines in the secretory granules. We have utilized cDNA probes for proenkephalin (PE) and for tyrosine hydroxylase (TH) to measure changes in specific mRNA content in response to depolarization of the cells with veratridine, in the presence or absence of dexamethasone. Veratridine treatment depleted both enkephalins and catehcolamines: PE mRNA and enkephalins began to recover after 24 hrs, and dexamethasone potentiated this recovery. TH and catecholamines were resistant to the effects of dexamethasone. Thus, co-transmitter synthesis need not be co-regulated.

The NG-108 cell line contains both enkephalin peptides and a δ-opiate receptor. We have used this line to study the effect of chronic exposure to an opiate agonist or antagonist on synthesis of opioid peptides. Antagonists are without effect but agonists such as etorphine or morphine up-regulate biosynthesis of proenkephalin. Two other transmitter systems do the same; muscarinic cholinergic and α-adrenergic. All three share one feature in common - their receptors inhibit adenylate cyclase - and we are currently investigating this relationship further. We have also initiated studies on PE expression in primary cultures of striatal cells, in order to extend our in vivo pharmacological studies to the molecular level. Detection of PE mRNA in these striatal cultures has required the development of a more sensitive hybridization protocol and a great deal of effort has been expended on this project. As a result, we are now using a random hexamer probe preparation which has increased the sensitivity of detection 40-fold. This increased sensitivity in hybridization technique has also been essential for studies on nerve growth factor (NGF) and related neurotrophic factors in brain. We are using for these studies the pcd mutant mouse, in which Purkinje cells die out, followed by a reactive gliosis. An NGF-like protein increases with the gliosis - we are currently trying to clone it using the NGF probe. This project should help explain the role which such neurotrophic factors may play in disease states.

Group on Receptor Pharmacology (Head: D.-M. Chuang, Ph.D.)

The research activity in Dr. Chuang's group has been focussed on the receptor regulation. Dr. Chuang has begun a collaboration with Drs. C. Malbon (SUNY at Stony Brook) and H. Alho (Fidia-Georgetown Neuroscience Institute) to develop an immunohistochemical method for the study of β-adrenergic receptor internalization which is an important step during agonist-induced receptor desensitization. Previously, Dr. Chuang has shown that internalized β-adrenergic receptors in a model system of frog erythrocytes are sequestered in endocytotic vesicles. Moreover, mechanisms involving coated pits and coated vesicles appear to be operating during this receptor internalization. In order to better understand the molecular details of this event, they have used antiserum raised against purified guinea pig lung β-receptor to label the receptor present in frog erythrocytes and found that 30-40% of the cells are specifically labeled by this antiserum. Moreover, after 2 hr receptor stimulation, the β-receptor labeling is more intense in loci which appear to be intracellular when compared with the control. Currently they are attempting to study the β-receptor morphology at the electron microscopic level in order to elucidate the molecular events of receptor internalization. Drs. Chuang and C. Carcia (St. Louis University) have also investigated whether β-receptor internalization occurs in the CNS. They have found that coated vesicles purified from bovine brain contain β-receptor binding sites which are uncoupled to the GTP binding protein and adenylate cyclase. These receptors can be labeled by a lipophilic ligand but not by a hydrophilic ligand, indicating that these sites are cryptic in nature and may be molecular entities undergoing internalization or exocytosis.

Drs. B. Roth (Guest Worker), T. Nakaki (Visiting Fellow) and Chuang continued their studies on the role of 5-HT_2 receptors in the contraction of rat aorta. They have shown that 5-HT_2 receptors in the aorta are linked to phosphoinositide-specific phospholipase C and that 5-HT induced contraction is a complicated scenario, involving receptor-mediated activation of a voltage sensitive calcium channel and phospholipase C. The 5-HT induced contraction and activation of phospholipase C are highly correlated. The tumor-promoting phorbol esters inhibit the 5-HT induced contraction and phospholipase C activation. Their results support the notion that phorbol esters desensitize the 5-HT_2 receptor-mediated response by protein phosphorylation through activation of protein kinase C (a calcium and phospholipid dependent kinase). Drs. Nakaki and Chuang have shown that the substrates for protein kinase C in a cell-free system of aorta are the myosin light chain and a protein of 92 K daltons. Currently they are extending these studies by using a primary culture of rat aorta smooth muscle cells in order to elucidate the physiological substrates for the kinase C following short term and long term stimulation of 5-HT_2 receptors.

Drs. B. Roth, S. McLean, (Lab. Neurophysiology, NIMH), X.-Z. Zhu (Visiting Fellow) and Chuang have characterized an unique 5-HT_2 receptors in the rat striatum. These receptor sites labeled by ^{3}H-ketanserin are resistant to the displacement by mianserin in autoradiographic receptor binding studies. They have proposed that these sites could be the receptor for a putative protein ligand (Mr=6000 daltons) which they have identified previously. They also have found that there exists two populations of ^{3}H-ketanserin binding sites in the rat striatum and that only the high affinity binding component is affected by GTP and can be up-regulated by chronic para-chlorophenylalanine treatment.

Dr. Chuang and Ms. Dillon-Carter (Chemist, LPP) have begun their studies at identifying and characterizing receptors linked to phospholipase C in NCB-20 cells, a neurohybrid between neuroblastoma and fetal brain cell. They have found that receptors of acetylcholine (M_1), histamine (H_1), bradykinin, neurotensin and angiotensin II are coupled to phospholipase C. The activation by these three neuropeptides may involve a common or convergent mechanism because their effects are non-additive in nature. Elucidation of the functional role of these receptor second messengers is now in progress. They also have found unexpectedly that the inositol-1-phosphatase in NCB-20 cells is about 10-time more resistant to the inhibition by lithium than in other systems. Since this enzyme is a possible site of action for the therapeutic effect of lithium on manic depression, their finding might have clinical implication for the lack of sensitivity of manic depressive patients to lithium treatment in some clinical cases.

Drs. Zhu and Chuang have studied the effects of differentiation of NCB-20 cells (induced by cyclic AMP or butyrate) on the expression of neurotransmitter receptors. They found that dibutyryl cAMP markedly decreases the density and biochemical response of the muscarinic cholinergic, adrenergic α_2 and opiate receptors. In contrast, butyrate increases 4-fold the binding sites for opiate receptors. Their results imply that cell differentiation may involve induction of new messenger RNA as well as disappearance of old messenger RNA for the receptor protein. Since NCB-20 cells express an unusually high density of imipramine binding site (which is a component of the 5-HT uptake system), Dr. Zhu has solubilized these sites from the cells and is developing useful procedures for further purification. Dr. Chuang, in collaboration with Drs. B. Schrier and E. Butler at NICHD have begun their attemp to synthesize cDNA complementary to the messenger RNA of NCB-20 cells in a final goal to construct a cDNA library for the cloning of imipramine binding site and other receptors.

Dr. J. Xu (Visiting Fellow) continued her studies on the regulation of receptors in the primary culture of cerebellum granule cells. Her previous studies showed that $GABA_B$ and muscarinic cholinergic receptors in granule cells are linked to the inhibitory adenylate cyclase through interaction with the Ni protein. Recently, Drs. Xu and Chuang showed that receptors for 5-HT, acetylcholine and norepinephrine are coupled to phospholipase C in cultured granule cells. The muscarinic receptor-mediated response is insensitive to a pertusis toxin but can be desensitized by preexposure to the receptor agonist with a concomitant loss of the receptor binding site. Further characterization of these receptor-mediated events may shed light on the regulation and role of the receptor-coupled phospholipase C in the CNS.

In collaboration with Drs. T. T. Quach, A.-M. Duchemin (Neuropsychiatry Branch, NIMH) and B. Schrier (NICHD), Dr. Chuang is involved in the cloning of nerve trophic or surviving factors induced by lesion with cortical aspiration of rat brain. They have succeeded in constructing a cDNA library for the messenger RNA induced by lesion, using a high efficiency expression vector. They have made good progress in screening for the bacterial clone that expresses the lesion-induced factors. Their study may lead to a better understanding of the role of nerve trophic or surviving factor in the repair process following brain injury.

Dr. Byrd (Guest Researcher) has continued the investigation of mechanisms of differentiation of neural crest cells, using the PC12 cell line as a model system. The differentiation promotor sodium butyrate is able to induce PC12 cells to differentiate into APUD-like cells, as contrasted to nerve growth factor (NGF), which induces these cells to differentiate into sympathetic-like neurons. The sodium butyrate-induced changes include cessation of cell division, marked morphological changes, and increased cellular adhesion. Studies performed in collaboration with Dr. Jose Naranjo (Fidia-Georgetown Institute for Neuroscience) and Dr. Iris Lindberg (L. S. U. Medical Center) have shown that butyrate-treated cells show a marked increase in the levels of proenkephalin mRNA, which is characteristically present in chromaffin cells, but absent in untreated PC12 cells. Processing of the proenkephalin gene product into smaller opiate peptides also occurs. These smaller enkephalin-containing peptides have molecular weights similar to those of the opioid peptides found in the adult chromaffin cell. Sodium butyrate also induces transglutaminase (TGase) activity in PC12 cells. In collaboration with Dr. Ulricke Lichti (NCI), Dr. Byrd has demonstrated that PC12 cells may express two types of TGase. The soluble form of the enzyme, the loss of which is associated with neoplastic transformation, is preferrentially induced by sodium butyrate. In contrast, NGF appears to induce only the particulate form of TGase. The time course for these two inductions is quite different. The sodium butyrate-induced increase in TGase activity is maximal by two days, while the NGF-induced activity increases for at least 21 days. A preliminary hypothesis explaining this data is that NGF activates a differentiation mechanism that is normally present in vivo, while sodium butyrate is partially reversing the transformed state of the cell through mechanisms as yet unknown. These two types of differentiation sequences are currently under investigation in our laboratory. In related studies done in collaboration with Dr. Maria Hadjiconstantinou, Dr. Byrd has explored the ability of PC12 cells to express the gene for phenylethanolamine N-methyltransferase (PNMT). PC12 cells have been reported to contain no epinephrine and no PNMT activity. However, using sensitive analytic methodology, it has been found that PC12 cells do actually synthesize small amounts of epinephrine, and that the content of intracellular epinephhrine is increased by treatment with dexamethasone. The increase in epinephrine is at least partially the result of increased PNMT activity in the cells. These studies show that the PC12 cells may therefore prove useful in the study of the initial expression of PNMT during embryogenesis.

Section on Neuropeptides (Chief: H.-Y. T. Yang)

The section on Neuropeptides has continued to study (1) roles of phe-met-arg-phe-NH_2 like peptides in analgesia, (2) role of NPY in adrenal function and (3) enkephalin metabolism.

Recently, several lines of evidence suggest that either phe-met-arg-phe-NH_2 (FMRF-NH_2) or FMRF-NH_2-like peptides may function as physiological opioid antagonists. FMRF-NH_2 is a neuropeptide of clam origin but FMRF-NH_2 like peptides are present in mammalian CNS. In earlier work, we have isolated and chemically characterized two peptides which are immunoreactive to the antiserum raised against FMRF-NH_2. These peptides, phe-leu-phe-gln-pro-gln-arg-phe-NH_2 (F-8-F-NH_2) and ala-gly-glu-gly-leu-ser-ser-pro-phe-trp-ser-leu-ala-ala-pro-gln-arg-phe-NH_2 (A-18-F-NH_2) differing substantially from the clam peptide FMRF-NH_2, can decrease basal tail flick latencies and F-8-F-NH_2 can also attenuate morphine induced analgesia when injected intraventricularly. In order to further explore the role of these putative endogenous opioid antagonists, antibodies against F-8-F-NH_2 and A-18-F-NH_2 were produced and distributions of these two peptides in brain and spinal cord were studied by both radioimmunoassays and immunohistochemical technique. The immunohistochemical study was carried out with the cooperation of Dr. Perti Panula at the University of Helsinki, Finland. These two peptides were found to be unevenly distributed in the brain with the highest concentrations in dorsal spinal cord and periaqueductal gray area and lowest levels in cortex and cerebellum. In the spinal cord, immunoreactivities were found in laminae I-II of the posterior horn. Immunohistochemically, several groups of neurons in the rat brain were detected; immunoreactive cells were found in the cerebral cortex, lateral septal nucleus, nucleus of the diagonal band, neostriatum, periventricular hypothalamic areas and several medullary nuclei. The identification of F-8-F-NH_2 and A-18-F-NH_2 in brain neurons suggests that these two peptides may function as neuromodulators or neurotransmitters. The enrichment of these two peptides in dorsal spinal cord and periqueductal gray area further supports our earlier working hypothesis that these two peptides may participate in opioid mediated pain modulation.

Our group, with the cooperation of Drs. P. Pizzo, A. Miser and A.-M. Alho at NCI, is also initiating a research program to study the putative endogenous opioid antagonist peptides in the blood and CSF of normal and opiate treated cancer patients. The goal of this study is to explore whether endogenous opioid antagonist peptides participate in development of opioid tolerance.

Neuropeptide Y (NPY) is present in high quantity in adrenal glands of various species. Although it is now known that NPY is stored in granules in norepinephrine containing chromaffin cells and also in nerve fibers crossing through the cortex and medulla, the role of

NPY in adrenal function still remains to be established. In earlier studies, Dr. Higuchi has found that NPY contents in adrenal glands of rats increase markedly with age during maturation and this increase is dependent on splanchnic nerve activity. In the older rat, in addition to the very large increase in the quantity, another molecular form of NPY was detected. Furthermore, it was demonstrated that NPY at low concentrations (1 nM to 200 nM) inhibited the nicotine induced release of both norepinephrine and epinephrine in a concentration dependent manner. The results of these studies imply that NPY may play a significant role in the regulation of catecholamine release from the adrenal gland during maturation or aging.

In order to explore whether adrenal NPY is released into circulation and then acts on distant peripheral target sites, we have studied the physiological release of NPY. Using retrogradedly perfused bovine adrenal glands, release of NPY was induced by acetylcholine and this effect was antagonized by hexamethonium. The results suggest that similarly to catecholamines, NPY secretion is mediated by the activation of cholinergic nicotinic receptor (the perfusion experiments were carried out in cooperation with Dr. T. Hexum at the University of Nebraska). The blood levels of NPY were determined in rats of various ages and also in adrenal demedullated rats. The adrenal NPY content was found to increase markedly with age, but this highly obvious change was not observed in plasma levels of NPY. The plasma NPY levels were also not significantly altered by the demedullation. These results suggest that adrenal glands are not the main source of circulating NPY and the change in adrenal NPY content during aging may have consequences on the release of catecholamines from adrenal medulla.

We are currently also purifying the NPY-like peptide, which appears in adrenal glands of older rats, in an attempt to characterize this peptide both biologically and biochemically.

In previous studies, we have demonstrated that met^5-enkephalin-arg^6-phe^7 is metabolized by a dipeptidyl carboxypeptidase and an aminopeptidase; furthermore Hoe 498 can inhibit the degradation of met^5-enkephalin-arg^6-phe^7 mediated by the dipeptidyl carboxypeptide with IC$_{50}$ value of 0.8 nM in vitro. In continuing this project, Dr. Mellstrom and Dr. Yang found that met^5-enkephalin-arg^6-phe^7 released from brain slices can be increased by Hoe 498. The drug also potentiates and prolongs the analgesic effect of intraventricularly administered met^5-enkephalin-arg^6-phe^7. With the availability of this highly potent inhibitors, the physiological role of met^5-enkephalin-arg^6-phe^7 may now be better studied.

The project of Dr. R. Rothman (Guest Worker) is directed towards testing a model of the opioid receptors sufficiently complex enough to explain varied physiological data. This model postulates a receptor complex consisting of adjacent and interacting mu, delta and kappa binding sites, as well as distinct mu, delta and kappa receptors not associated with the receptor complex.

To test this model, we utilized several techniques: (1) quantitative ligand binding studies using the method of "binding surface analysis" and weighted nonlinear least squares curve fitting, (2) site-directed alkylating agents such as BIT (mu-directed), FIT (delta-directed) and beta-FNA (mu-directed), (3) receptor autoradiography to provide anatomical information, (4) "in vivo" manipulations such as chronic morphine and chronic naltrexone which up-regulate opiate receptors and (5) biochemical information using a technique to cross-link ^{125}I-beta endorphin to opiate receptors.

As our work progress, not only is the model tested, but additional data is generated. Our work addresses fundamental issues of morphine tolerance and dependence. It has defined the opiate receptors labeled by (^{3}H) cycloFOXY, a novel antagonist suitable for positron emission topography. We have developed methods for measuring rat brain kappa receptors, a subtype of the opiate receptor implicated in eating disorders and work is underway to develop a kappa-directed site-directed alkylating agent.

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